C L I N I C A L A S P E C T S O F R H E S U S

I S O I M M U N I Z A T I O N

B Y

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March, 1971.

In the preparation of a Thesis encouragement and guidance play a large part. In this I have been very fortunate. I am happy to acknowledge the assistance of the following:-

DR. JOHN WALLACE, B.Sc., M.D., F.R.C.Path., M.R.C.P.(G.) for encouragement and helpful criticism.

MR. GEORGE MILNE, F.P.S. for advice on the study of liquor amnii.

The Technical Staff of The Regional Transfusion Service who examined the routine blood samples.

The Scientific Staff of The Regional Transfusion Service who examined the routine liquor amnii.

MISS MARIAN M. IZZAT, M.Sc. for help with statistics.

DR. MALCOLM D. BLACK, F.R.C.S.E., F.R.C.O.G., F.R.C.P.(G.) for helpful criticism and allowing me to undertake the clinical care of his patients.

DR. ROBERT A. TENNENT, O.B.E., F.R.C.O.G., F.R.C.S.(G.) for allowing me to study the patients in his care.

DR. W.R. McAINSH, M.R.C.P.(E.) who also allowed me to study his patient

DR. W.H. DEMPSTER, D.M.R.D. for radiological assistance.

MISS ANN CLARKE for secretarial assistance.

C O N T E N T S

INTRODUCTION

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The object of this thesis is to describe the modern management of the problem of Rhesus isoimmunization. An historical review and a description of the material used from a County Obstetric Service is given.

The management is considered under three main headings, namely:- assessment of severity based on liquor amnii examination; treatment of the severely affected foetus by intra-uterine transfusion and, the recent and considerable advance in the prevention of isoimmunization.

In the section on liquor amnii examination a cord blood factor as an indicator of the degree of severity of the Haemolytic Disease at birth is suggested by the author and correlated with liquor amnii prediction.

CHAPTER 1

H I S T O R I C A L R E V I E W

HISTORICAL REVIEW

For many years clinicians were puzzled by the condition known as erythroblastosis foetalis, now known as Haemolytic Disease of the Newborn. There was also lack of knowledge about haemolytic reactions following the transfusion of ABO compatible blood to certain patients, particularly marked if these same patients were further transfused with ABO compatible blood. The unity of hydrops foetalis, icterus gravis and congenital haemolytic anaemia was established (Diamond, Blackfan and Baty, 1932) but there was no obvious explanation why some marriages, with no apparent disease or ABO blood group abnormality in either party should result in a succession of diseased or stillborn infants showing signs of one of the three aforementioned conditions. In 1939 Levine and Stetson described an atypical immune agglutinin in the serum of a woman after delivery of a stillborn infant showing hydrops foetalis and this was the first in a series of rapid developments which led up to the present concept of the Rhesus blood group and its clinical effects.

In 1940 Landsteiner and Wiener reported the findings of a hitherto unrecognised antigen by testing human red cells against an anti Rhesus serum prepared by injecting blood from Rhesus monkeys into rabbits or guinea pigs. This they termed the Rhesus antigen and noted it occurred in the erythrocytes of 85% of Europeans.

Later in the same year Wiener and Peters (1940) showed the presence of a Rhesus antibody in the blood of Rhesus negative patients who had suffered haemolytic reactions following transfusion with Rhesus positive blood. Levine, Burnham, Katzin and Vogel

(1941) proved that the formation of Rhesus antibody by a Rhesus negative woman frequently resulted in stillbirth or one of the syndromes grouped together under the term "erythroblastosis foetalis". They suggested that the formation of antibody was the maternal response to immunization by the Rhesus antigen derived from the foetus which had inherited it from its Rhesus positive father.

When all the variations of the Rhesus blood system became known it was realised that individuals could have one or two Rh. (D) genes in their blood. The presence of one Rh. (D) gene made an individual Rhesus positive and, in that circumstance, there was a 50% chance of this antigen being passed on by such a father to his offspring, producing a risk of Haemolytic Disease in the child. Such an individual is said to be heterozygous Rh. (D) positive. Where an individual has two Rh. (D) genes in his genetic make-up and fathers a child, he must pass on one Rh. (D) gene to his child with the risk, therefore, of producing Haemolytic Disease in each child. Such an individual is said to be homozygous Rh. (D) positive. The details of these and the other classification of the Rhesus group are well described by Race (1948).

It became evident from this early work that routine testing for the Rhesus factor both in transfusion matchings and antenatal screening was essential. In the early years of testing sera for the presence of anti Rh. (D) agglutinins it was soon recognised that anti Rh. (D) antibodies could not be demonstrated in the sera of many mothers of babies clinically proven to have erythroblastosis foetalis. Up to 1944 anti Rh. (D) antibodies were demonstrated by incubating a range of cells suspended in saline or citrate with the unknown serum for two hours at 37°C. (Levine, 1943a).

The explanation of these anomalies came simultaneously and independently from Race (1944) in England and Wiener (1944) in America. They showed that the anti Rh. (D) antibodies occurred in two forms - a blocking and an agglutinating type and that the former was capable of inhibiting the agglutination of Rh. positive cells by the latter. Race (1944) gave the name incomplete to this type of antibody. Later in 1945 Coombs, Mourant and Race showed that cells sensitized with an incomplete or blocking antibody and washed free of other non-specific proteins would show agglutinations after the addition of an anti-human-globulin serum. This anti-human-globulin serum was prepared by the immunization of rabbits with whole human serum or globulin or pseudoglobulin fractions. They were all equally suitable as the test reagent after they had been absorbed with a mixture of washed cells of Groups A, B and O.

With the knowledge that two types of antibody - a saline antibody and a blocking or incomplete antibody - existed and, that the latter could be missed without adequate testing, it became obvious that both tests had to be carried out routinely in antenatal blood tests and transfusion matchings.

With the elucidation of the cause of Haemolytic Disease came the early attempts at treatment. Initially when a pregnancy was diagnosed and the foetus suspected of having Haemolytic Disease three lines of action were considered:-

(1) Induction of abortion
(2) Induction of premature labour
(3) Allowing the pregnancy to go to term

In (2) and (3), preparations were made to transfuse the baby on delivery if necessary.

INDUCTION OF ABORTION

This was rarely considered and then only where there was a previous stillbirth due to isoimmunization and the father was known to be homozygous. Termination of pregnancy in these cases was carried out in the first trimester.

INDUCTION OF PREMATURE LABOUR

This was suggested by Diamond (1947). It was appreciated that this imposed the additional risk of prematurity on these infants but did improve the infants chance of recovery from Haemolytic Disease. Diamond (1947) suggested that induction was best carried out at 37-38 weeks but Mollison (1948) mentions 35 weeks in the case of a woman who has had one or more severely affected infants. Action was only taken when it was virtually certain, or extremely probable, that the foetus was severely affected. Planned induction of labour was based on the presence of isoimmunization in the mothers with evidence of increase in sensitization in the pregnancy, indicated by a rise in antibody titre, or a previously affected child and homozygosity of the husband.

THE TREATMENT OF THE AFFECTED INFANT

The treatment of the affected infant was either a simple transfusion or the then new exchange technique of Diamond (1947). A decision to transfuse or not was taken largely on clinical examination plus examination of the cord blood. The most important findings were regarded as the rapid onset of jaundice and a haemoglobin of

12 gm.% or less. When the condition of the baby was judged as severe by these standards an exchange transfusion was performed soon after delivery. In milder cases a simple transfusion, either via the umbilical vein or a scalp vein, was given. Over the years these techniques have been mastered and improved.

At this time most research was applied to the amelioration of the effects of Haemolytic Disease on the foetus. Of the early attempts to improve foetal results one of the best known is that of Carter (1947) who introduced the use of Rh. Hapten. A Hapten is a substance which, when combined with a suitable carrier such as protein, can stimulate antibody production in a host and can also react specifically with that antibody, but which without a carrier cannot by itself initiate antibody production. Carter's rationale in the use of the Rhesus Hapten was to neutralise the existing antibody in a patient without stimulating the production of more antibody. The Hapten was given initially by intramuscular injection in large amounts but in 1958 Carter and Lewis published a paper giving successful results in 17 sensitized patients using the Hapten orally. One of the essentials of Carter's treatment was that the Hapten had to be given between pregnancies and during pregnancy.

As Rh. Hapten was prepared from freely available time expired red cells it is surprising that no other worker confirmed the value of this substance.

A further attempt to prevent damage to the foetus of the sensitized mother was the use of corticosteroids. The rationale in their use was the hope that the production of the maternal antibody might be suppressed and that severity and rapidity of destruction of the foetal cells might be lessened. Opinion on the use of corticosteroids was

divided. Hunter (1954) claimed that they were of value but De Costa, Gerbie and Potter (1954) disagreed as did Wiener (1954). The use of corticosteroids was shortlived as their value, if any, was minimal and their use carried some risk to the mother.

A modern attempt to protect the foetus by reducing the level of circulating maternal antibody and thereby reducing the potential effect on the foetus is by the use of plasmapheresis in pregnancy. This process involves the removal of large quantities of blood by venesection, removing the plasma and returning the red cells to the patient. This has been shown to reduce the antibody level but the effect is transient and the titre rises to its original level in a few weeks (Powell, 1968; Clarke, Elson, Bradley, Donohoe, Lehane and Hughes Jones, 1970).

These measures will be discussed more fully when the problem of prevention of isoimmunization is discussed in detail.

While these problems were being investigated other aspects of Haemolytic Disease were also receiving attention. It had been recognised by Pickles (1949) that the liquor amnii in a case with a badly affected foetus was golden in colour. Bevis in 1953 examined the contents of liquor amnii and in 1956 the relationship of liquor bilirubin and the later development of kernicterus. It was realised from his work that amniocentesis was a relatively simple procedure and several workers began to study liquor carefully with particular reference to the "bilirubin pigment" content.

Following the lead of Bevis many workers became interested in the bilirubin pigment content of liquor amnii as a means of prediction

in Haemolytic Disease. From the publication of a paper by Walker (1957) to the present time a great deal of experience has been accumulated on technique and dangers of amniocentesis, methods of examination of the liquor and correlation between liquor prediction and outcome of the pregnancy. Of the many contributors - Mackay (1961); Alvey (1964); Walker, Fairweather and Jones (1964); Stewart, Taylor and Beck (1967) and Freda (1965) to name only a few, the most outstanding contribution has come from Liley, (1961). He rationalised the whole problem by producing from his own work a graph which related liquor findings to severity of affection of the foetus at different stages of gestation.

Using spectrophotometry to examine the liquor amnii in a large series of sensitized patients, Liley was able by correlating the liquor prediction to the outcome of the pregnancy to divide his liquor prediction into three zones - Upper, middle and lower. The lower zone contained results which indicated that the baby would be mildly affected or unaffected. The middle zone contained results which indicated that the baby would be moderately affected, probably needing an exchange transfusion, and the upper zone contained results which indicated that the baby would be badly affected. These three zones Liley superimposed on a semilogarithmic graph using the optical density of liquor on a logarithmic scale as the ordinate and the maturity in weeks was plotted as a simple scale on the abscissa.

Most other workers since then have used Liley's graph either in its original form or with some modification, as a basis for their own series. From the early nineteen sixties, examination of liquor amnii has played an important part in the antenatal prediction of severity of Haemolytic Disease and the stage has now been reached

that, with the use of liquor predictions, the disaster of very early induction of a Rhesus negative baby should no longer occur.

In 1961 Liley made a major contribution to the Rhesus problem by his work on liquor amnii but in 1963 he made a further important contribution in the field of active treatment of the foetus endangered by Haemolytic Disease, by introducing intra-uterine transfusion. This meant that once a foetus was judged to be severely affected by Haemolytic Disease active treatment could be instituted. Liley, having found how relatively simple it was to enter the peritoneal cavi of a hydropic foetus during amniocentesis, remembered the fact that blood could, in the young, be absorbed into the circulation from the peritoneal cavity and put this into therapeutic use. Many other workers have followed his example (Karnichi, 1966; Friesen, Bowman, Barnes, Grewar, McInnis, Bowman (1967); Fairweather, Tacchi, Coxon, Hughes, Murray, Walker, 1967) and the use of intra-uterine transfusion is now an accepted practice. The procedure does not salvage the baby in all cases and is not without risk to mother or foetus but it gives some hope to the patient with a history of repeated stillbirths and its introduction was a landmark in the history of Haemolytic Disease.

While these clinical advances were being established, work on the problem of prevention of formation of antibodies was being tackled with renewed vigour. Levine (1943) suggested that ABO incompatibility between mother and foetus afforded some protection against Rhesus immunization. Race and Sanger (1950) suggested that isoimmunization was probably due to the passage of foetal cells into the maternal circulation and that the ABO incompatible cells of the foetus were destroyed in the maternal blood stream by naturally occurring anti A and anti B agglutinins before sensitization by the foetal cells could occur.

Experimental work by Stern, Davidsohn and Masaitis (1956) using Rhesus negative male volunteers supported the idea of protection by ABO incompatibility. They injected Rhesus positive blood into Rhesus negative male volunteers and noted that much higher titres of anti Rh. (D) were obtained when compatible ABO blood was injected than when incompatible blood was used.

Clarke, Finn, McConnell and Sheppard (1958) carried out family studies to test Levine's theory of ABO protective mechanism and found that in 14 of 23 families where the parents were ABO incompatible the sensitizing foetus was ABO compatible with the mother.

Almost simultaneously work was going on to establish the fact and importance of transplacental haemorrhage from baby to mother. In 1957 Kleihauer, Hildegard and Betke demonstrated the presence of small numbers of foetal erythrocytes in maternal circulation using his own staining technique. Zipursky, Hull and White (1959) and later Finn, Clarke, Donohoe, McConnell, Sheppard and Lehane (1961), using the Kleihauer technique, showed that an appreciable transplacental haemorrh occurs during the third stage of labour.

Kreiger (1966) showed conclusively that transplacental haemorrhage may also occur during pregnancy.

Having established that maternal sensitization was probably caused by small "transfusions" from a Rhesus positive foetus, transplacental haemorrhage was then investigated in detail by Chown (1954); Kleihauer et al (1957); Finn, Clarke, Donohoe, McConnell, Sheppard, Lehane (196 Cohen and Zuelzer (1964); Cohen and Zuelzer (1967) and Woodrow, Clarke Donohoe, Finn, McConnell, Sheppard, Lehane, Russell, Kulke and Durkin

(1965) with particular reference to:-

(1) Incidence of transplacental haemorrhage
(2) Correlation between circulating foetal cells and the production of Rhesus antibodies
(3) Time of foeto-maternal haemorrhage
(4) Association with obstetric manoeuvres during pregnancy and delivery

Simultaneously with these investigations experimental work was going on in Liverpool to investigate the effect of injecting Rhesus negative male volunteers with Rhesus positive blood and treating half of them with plasma containing anti Rh. (D) (Finn et al, 1961). The results of this experiment suggested that anti Rh. (D) played an effective part in rapidly removing Rhesus positive cells from the circulation thus preventing the formation of antibodies. Clarke, Donohoe, McConnell, Woodrow, Finn, Krevans, Kulke, Lehane and Sheppard (1963) showed also experimentally in Rhesus negative male volunteers that the injection of plasma containing the saline anti Rh. (D) antibody following the injection of Rhesus positive blood enhanced antibody formation.

During this experimental work, plasma containing anti Rh. (D) was used and all workers were aware of the danger of transmitting homologous serum jaundice by the use of plasma. In 1964 Freda, Gorman and Pollack produced a gamma globulin fraction which does not carry the risk of transmitting homologous serum jaundice, prepared from the plasma of donors each of whom had a high titre of anti Rh. (D) by the indirect antiglobulin method. This fraction was prepared by a modification of the Cohn fractionation technique and sterilized. This was used experimentally on Rhesus negative male volunteers with similar results to that obtained by the Liverpool workers.

These early experiments were all carried out on male volunteers. There was no reason to suppose that the Rhesus negative female would react differently to the male when injected with Rhesus positive blood. Woodrow et al (1965) injected Rhesus positive foetal cells into 10 Rhesus negative nulliparous postmenopausal women and gave anti Rh. (D) gamma globulin. They showed that the Rhesus positive foetal cells could be cleared from the circulation of these women as effectively as the adult Rhesus positive cells could be cleared from the adult male Rhesus negative volunteers.

The first clinical trials of anti Rh. (D) gamma globulin were carried out in 1966 in several centres in England and Baltimore. The gamma globulin was given in 5 ml. amounts only to patients who delivered a Rhesus positive ABO compatible baby and was given in the first 36 hours after delivery (A Combined Study, 1966).

In this combined study 78 patients were given anti Rh. (D) gamma globulin and 78 controls were not. Of the 78 patients treated none were found to be immunized at an interval of six months or later after delivery but 19 of the 78 controls were immunized at a similar interval of time. In a series reported by Clarke (1967) with results from various centres with a total of 1,227 women in the study, 75 out of 559 controls were found to have antibodies six months or later after delivery and only one out of 628 treated women developed antibodies in the same period of time.

The amount of gamma globulin given was 5 ml. but the actual amount of anti Rh. (D) gamma globulin was not known. With improved techniques of measurement (Hughes Jones, 1967) the dose can now be measured in microgrammes. The average dose used in Rhesus negative women who

deliver Rhesus positive ABO compatible children is now 200 micrograms. This has been available for such women in a first pregnancy since March, 1968. It may be that a smaller dose will suffice except in the case of a massive transplacental bleed. Further trials, as yet unpublished, are now taking place to try and establish the minimal effective amount of anti Rh. (D) gamma globulin required.

Scarcity of raw material and hence gamma globulin has made a choice of priorities essential. The anti Rh. (D) gamma globulin was given initially only to Rhesus negative primigravid women who delivered a Rhesus positive ABO compatible baby and who did not have anti Rh. (D) in their serum.

At the moment, most raw plasma containing anti Rh. (D) comes from women who have been sensitized by pregnancy or by an incompatible blood transfusion but this will not be adequate. To augment the supply of raw material plasma is being obtained from postmenopausal isoimmunized women who have been given booster doses of Rhesus positive cells and from Rhesus negative male volunteers who have been immunized by the injection of Rhesus positive blood. This as yet has only been done in certain parts of the country on a small scale but the programme will have to be extended to ensure adequate supplies of anti Rh. (D) gamma globulin for the future.

The process of plasmapheresis (see Page 156) has been developed with these donors. Fairly large amounts of plasma can be obtained at one time from each donor on a planned programme and the donors are able to undergo plasmapheresis more often than giving a simple donation of whole blood.

Since the efficacy of anti Rh. (D) immunoglobulin has been established large amounts of anti Rh. (D) containing plasma are now required. This is most easily obtained by the use of plasmapheresis of suitable donors.

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CHAPTER 11

MATERIAL AND ORGANISATION

MATERIAL AND ORGANISATION

The material for this thesis was acquired during the eleven year period 1958-1968 in the County of Lanark. The work was carried out at Bellshill Maternity Hospital which, until 1962 contained 112 beds and 24 cots for sick and premature babies. In 1962 a new hospital was opened and this contained 132 beds and 56 cots for sick and premature babies. In conjunction with Bellshill Maternity Hospital work was carried out at The William Smellie Memorial Hospital, Lanark - a 50 bedded hospital serving the southern part of the county. These two specialist hospitals between them dealt with 5,000 deliveries which included the abnormal cases from the remaining 5,000 patients delivered in the county, per annum.

During the development of the specialised care of the Rhesus patients in Lanarkshire I have been allowed to play a unique part. From 1956 to 1964 I was employed as a Neonatal Paediatrician at Bellshill and the other maternity hospitals in Lanarkshire with the additional responsibility of two antenatal clinics. I rapidly developed an interest in the Rhesus problem and I was encouraged by the Senior Obstetricians to start the Rhesus clinic. As a result I had both antenatal and postnatal care of the babies which meant I could maintain continuity with each mother during the critical neonatal period when exchange transfusion might be necessary. When the regular meetings at The Regional Transfusion Centre started I was responsible for the collection of the clinical data and attended these meetings with my senior colleagues.

In 1964 I moved to a post in The Regional Transfusion Centre where I developed an interest in the laboratory investigation of these patients. I have been fortunate, however, in that I have

retained an active part in the clinical care of the Rhesus patients including carrying out amniocentesis and intra-uterine transfusion.

Since anti D gamma globulin was first used in 1967 in this area in prevention of isoimmunization I have taken a co-ordinating role in the organisation and record keeping of this work. I have also been the Medical Officer in charge of plasmapheresis for the collection of material for processing for anti Rh. (D) gamma globulin.

The policy of management of the Rhesus isoimmunized pregnant women in the late nineteen fifties in Lanarkshire was that of induction at 38-39 weeks with an occasional early Caesarean Section about 36 weeks if there was a history of previous stillbirth and a homozygous husband. Specimens of blood were taken from antenatal patients attending one of the sixteen clinics throughout the county at the first visit and then at 28 weeks. If antibodies were found at 28 weeks specimens of blood were examined thereafter at fortnightly intervals. If antibodies were discovered at the first visit, specimens of blood were examined at monthly intervals until 28 weeks and then at fortnightly intervals thereafter. When possible blood was obtained from the patients' husbands for phenotyping.

The decision to induce or otherwise was made by the individual obstetrician in charge of each antenatal clinic with the exception of the difficult case with a poor past history when reference was made to one of the senior consultants. The decision when to terminate a pregnancy was on antibody titre alone. Superficially this seemed to be a reasonable arrangement but because of the widespread area covered by the numerous antenatal clinics and the fact that specimens of blood were not examined in the hospital but in a laboratory some miles away there was occasional unavoidable delay in a clinical decision being made on a particular patient with unfortunate results.

In 1960 it was decided to establish a special antenatal clinic for the Rhesus sensitized patient both at Bellshill Maternity Hospital and The William Smellie Memorial Hospital at Lanark. This enabled patients from both halves of the county to be centralised at one of two places. A policy of induction at 38 weeks generally and earlier induction of specific patients was decided upon. This decision was based on antibody titres, previous history and genotype of husband.

An immediate effect of the establishment of these clinics was that the patients felt that their special problems were being actively treated, particularly as a close liaison was maintained with the paediatric unit. This was particularly true of patients who had previously had a baby transfused. All patients were referred to the special clinic as soon as antibodies were detected in their blood at routine antenatal clinics. With standardisation of antenatal care and induction, the outcome of pregnancy in the sensitized woman began to improve. This is illustrated in Table 11.

Still, however, there remained the problem of a woman with a heterozygous husband, a previously affected baby and a rapidly rising titre of antibody. After delivery of one of these women at 37 weeks of a Rhesus negative baby it was decided to look at the problem more closely. This was helped enormously by the fact that a short time previously The Regional Blood Transfusion Centre had moved to Law Hospital situated roughly halfway between Bellshill Hospital and The William Smellie Hospital and all antenatal and cord bloods were examined there. At the invitation of The Regional Director a meeting at regular intervals was established at The Transfusion Centre to discuss the sensitised cases. At each of these meetings, held monthly, all the clinical and laboratory facts

and figures were made available. The meetings, now held at three-weekly intervals, were attended by The Regional Director of The Transfusion Service and his deputy, a Consultant Obstetrician and a Paediatrician. As the number of cases increased with time, a wealth of experience was acquired from these meetings. A close liaison formed between the clinicians and the laboratory staff of The Regional Transfusion Service. This liaison became even closer with the introduction of amniocentesis since the liquors were examined at The Regional Transfusion Centre and another parameter was added to the consideration of each case.

Figure 1 illustrates the organisation. Sixteen routine antenatal clinics referred their cases to the special Rhesus clinic. Cases were discussed at The Regional Transfusion Centre and eventually admitted to hospital with a firm decision as to their management.

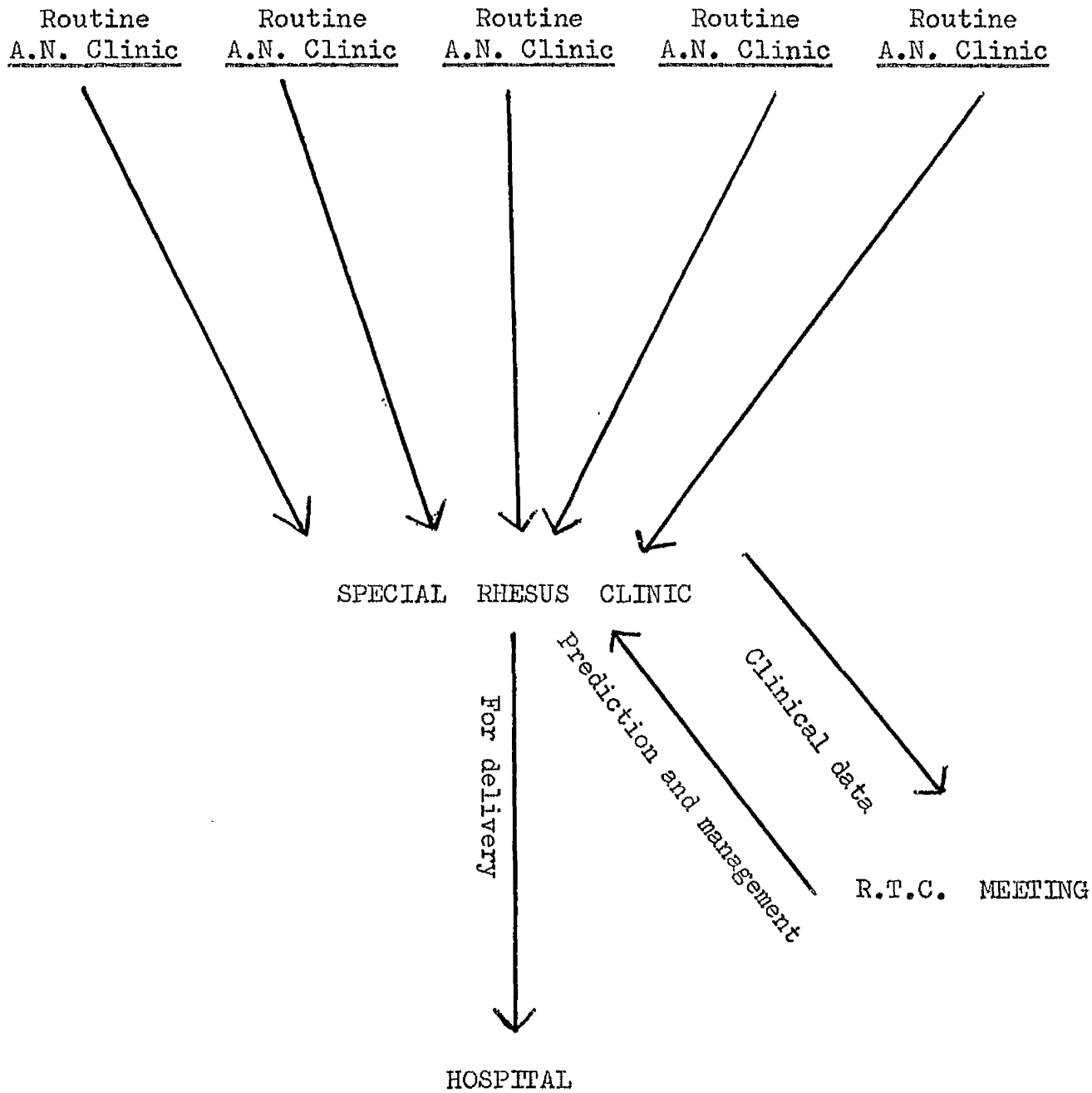
In summary, the evolution of the present day management of the Rhesus sensitized patient in Lanarkshire has been as follows:-

(1) The establishment of special Rhesus antenatal clinics within the Specialist Maternity Hospitals of the County.

(2) In association with the clinics, a regular three-weekly meeting was established to discuss the cases and make a decision on time and method of delivery of the patients.

(3) The introduction of the more sophisticated techniques of antenatal prediction and management in addition to (1) and (2) above.

FIGURE 1



KEY A.N. - Antenatal R.T.C. - Regional Transfusion Centre

The total number of sensitised cases dealt with in the period 1958-1968 was 917. The total number of hospital deliveries in this period was 55,000 and the total number of deliveries in the County of Lanarkshire was 110,000. The percentage of sensitised women in the hospital patients was, therefore, small (1.7). As the Lanarkshire Maternity Hospitals cared for most of the cases requiring specialist care in the County and, in particular, after 1960 when the Rhesus clinic was established, it can be safely said that all the sensitised cases in the County were confined in one or other of the specialist hospitals and thus the percentage of sensitised cases in the county was 0.8.

For convenience these cases have been divided into three broad groups:-

(1) Pre Rhesus clinic group when the care of sensitised patient was incorporated into general antenatal care and the only policy was one of induction of labour at 38 weeks.

(2) Rhesus clinic group when these cases were brought together and looked after by one person and a formula for management evolved.

(3) Rhesus clinic and amniocentesis group when the policies of management begun by the clinic continued in association with the more advanced forms of investigation.

The following tables are included to show in more detail the type of material and the overall results.

TABLE 1

TABLE SHOWING TYPE OF ANTIBODY

	Total	D	C+D	E	$\bar{c}$	D+E	$\bar{c}$+E	C+D+E	C
Pre Rh. clinic 1958 - 1959	121	87	24	4	2	3	1	0	0
Rh. clinic 1959 - 1965	489	362	88	13	12	5	6	2	1
Amniocentesis 1966 - 1968	307	200	64	16	15	3	6	2	C+$\bar{e}$ 1

The relative frequency of antibodies provoked by pregnancy is not the same as that provoked by transfusion. So far as anti D is concerned the incidence from transfusion is now very small since adequate precautions are now taken before a transfusion is given to a Rhesus negative recipient.

In a series by Giblet (1964) quoted by Mollison the commonest occurring antibody was anti D followed by anti C+D, anti E and anti $\bar{c}$. This is borne out by the incidence of antibodies in the 917 cases reviewed.

TABLE 11

TABLE SHOWING PERINATAL MORTALITY AS % OF TOTAL LIVE AND STILLBIRTHS

	Total Cases	Erythroblastosis S.B.	Erythroblastosis N.N.D.	Other causes S.B.	Other causes N.N.D.	Mortality All cases	Mortality Corrected
Pre Rh. clinic 1958 - 1959	121 Twins 2	8	6	5	1	16.3	11.4
Rh. clinic 1960 - 1965	489 Twins 12	33	13	6 (2)	16 (1)	13.6	9.2
Amniocentesis 1966 - 1968	307 Twins 3	7	10	5 (4)	3 (1)	8.1	5.5

KEY S.B. - Stillbirth N.N.D. - Neonatal death

The figures in this table include the Rh. negative babies which in addition are shown separately in brackets.

This table shows the perinatal mortality as a percentage of total live and stillbirths of the series. The steady improvement in results is a reflection of the more careful control and development of more sophisticated techniques in management of these cases.

TABLE 111

TABLE SHOWING OTHER CAUSES OF PERINATAL MORTALITY

		A.P.H.	R.D.S.	F.A.	Inf.	I.C.H.	P. Cord	Unexp.
Pre. Rh. clinic 1958 - 1959	S.B.	2	-	-	-	-	-	3
	N.N.D.	-	-	1	-	-	-	-
Rh. clinic 1960 - 1965	S.B.	2 (1)	-	-	1	-	-	3 (1)
	N.N.D.	-	10 (1)	3	2	1	-	-
Amniocentesis 1966 - 1968	S.B.	-	-	-	-	-	1 (1)	4 (3)
	N.N.D.	-	3 (1)	-	-	-	-	-

KEY

S.B. - Stillbirth
A.P.H. - Antepartum haemorrhage
F.A. - Foetal abnormality
P. cord - Prolapsed cord
Unexp. - Unexplained
Inf. - Infection
N.N.D. - Neonatal death
R.D.S. - Respiratory distress syndrome
I.C.H. - Intracranial haemorrhage

The figures in this table include the Rh. negative babies which in addition are shown separately in brackets.

This table shows a breakdown of causes of perinatal mortality in the series due to other than erythroblastosis.

TABLE IV

TABLE SHOWING RH. GROUP OF BABY

	Total Cases	Rh. Group +ve	Rh. Group -ve
Pre Rh. clinic 1958 - 1959	118 Twins 2	98	22
Rh. clinic 1960 - 1965	485 Twins 12	436	61
Amniocentesis 1966 - 1968	307 Twins 3	237	73

N.B. Total cases in this table less as baby's cord blood occasionally not available e.g. in case of macerated stillbirth

This table shows the incidence of babies which were Rhesus negative and Rhesus positive in the series.

TABLE V

TABLE SHOWING TREATMENT OF BABY

	No treat	1 exch. ± top up	2 exch. ± top up	3 exch. ± top up	Top up	S.B. or N.N.D. and No treat
Pre. Rh. clinic Twins 2	46	40	8	0	11	18
Rh. clinic	202	197	28	11	10	53
Amniocentesis Twins 3	158	82	27	18 *	5	20

* includes 3 babies with 4 exchange transfusions

KEY Exch. - exchange transfusion
S.B. - stillbirth
Top up - simple transfusion
N.N.D. - neonatal death

This table shows the treatment of the babies. The increased incidence of multiple exchange transfusions is a reflection of the increased survival of the more severely affected babies.

TABLE VI

TABLE SHOWING COMPATIBILITY OF FATHER'S ABO GROUP WITH MATERNAL GROUP

MOTHER	COMPATIBLE				INCOMPATIBLE				TOTAL
	O	A	B	AB	O	A	B	AB	
O	187	-	-	-	-	70	24	1	282
A	129	67	-	-	-	-	9	3	208
B	38	-	7	-	-	22	-	-	67
AB	3	1	1	1	-	-	-	-	6
TOTAL	357	68	8	1	-	92	33	4	563
TOTAL	434 = 77.1%				129 = 22.9%				

This table shows the incidence of ABO compatibility of the parents. The results bear out Levine's observation that there is a deficiency of ABO incompatible matings - 77.1% compatible as against 22.9% incompatible.

The figures for a random group of matings is 66% compatible and 34% incompatible (Mollison, Mourant and Race, 1948).

TABLE V11

TABLE SHOWING GRAVIDITY OF MOTHER

	Total Cases	1	2	3	4	5	6	> 6
Pre Rh. Clinic 1958 - 1959	121	1	27	31	22	10	16	14
Rh. Clinic 1960 - 1965	489	2	100	124	85	52	35	91
Amniocentesis 1966 - 1968	307	1	63	76	53	43	29	42

From this table it will be seen that a reasonably high proportion of women had more than three pregnancies. This naturally increases their chances of having badly affected babies and consequently such measures as intra-uterine transfusion may have to be used more often than in a population where the parity seldom rises beyond two or three.

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CHAPTER 111

A M N I O C E N T E S I S

A M N I O C E N T E S I S

The association of yellow amniotic fluid and hydrops foetalis has been appreciated since 1949 (Pickles) but only since the initiation of the investigation of liquor amnii by spectrophotometry and biochemistry by Bevis (1953, 1956) has it been realised how much valuable guidance in antenatal prediction could be obtained from the study of it. Bevis was originally concerned with the contents of liquor amnii with particular reference to the breakdown of haemoglobin and he tried to relate the concentration of non-haematin iron to the severity of Haemolytic Disease of the Newborn. He measured various substances present in liquor and his general conclusion was that an increase in the non-bilirubin icteric index was followed by kernicterus.

Since Bevis's work many others have investigated the constituents of liquor amnii in relation to Erythroblastosis Foetalis. Its potential as a valuable guide to the severity of the disease and to antenatal management is now realised. As might be expected, the method has limitations but to date it is considered to be the best single diagnostic aid.

BILIRUBIN CONTENT OF AMNIOTIC FLUID

Two methods of the assessment of the bilirubin content of amniotic fluid have been used.

These are:-

A. USING SPECTROPHOTOMETRY

This is used in two ways:-

(1) By grading according to the shape of the spectral absorption curve by visual inspection (Walker, 1957; Carey, 1960; Mackay, 1961).

(2) By optical density difference measurements obtained from the spectral absorption curves (Liley, 1961; Watson, 1962; Robertson, 1964; Knox, Fairweather and Walker, 1965; Alvey, 1964).

B. CHEMICAL ESTIMATION

This is claimed by some workers to be more accurate than spectrophotometry (Beecham, Molthan, Boutwell and Rohrbeck, 1962; Watson, 1962; Mackay and Watson, 1962; Bevis, 1953; Stewart and Taylor, 1964; Stewart, Taylor and Beck, 1967; Walker, Landon and Oxley, 1969), but in view of the very small amount of bilirubin being measured these techniques require considerable expertise in biochemical methods.

In this series the spectrophotometric method has been used as the examination of choice but in some instances both methods have been carried out. It is felt, however, that spectrophotometry gives the more accurate result (Milne, 1966).

It is not entirely certain if the substance which colours the liquor is bilirubin but it is certainly a substance which bears a close resemblance to bilirubin on examination by biochemical and spectrophotometric methods and for practical purposes it is assumed to be bilirubin. The route by which bilirubin reaches the liquor is not yet known nor to what extent its level is dependent on other constituents or turnover rate of the liquor. This does not prevent an empirical evaluation of bilirubin like substance in the liquor being of clinical significance.

TECHNIQUE OF AMNIOCENTESIS

Amniocentesis can be done at an out-patient clinic provided an aseptic technique is maintained. It causes little discomfort to the patient and when the purpose of the test is explained patients are most co-operative.

The patient lies comfortably on an examination couch for several minutes to allow a mobile foetus to settle and the foetal position is then carefully palpated and a decision taken as to the point of entry. Liquor can be most easily obtained from the area over the foetal limbs, from behind the foetal neck, from below the presenting part and occasionally from above the foetus near the fundus of the uterus. In practice the optimum point of entry

is often below and to the left or right of the umbilicus.

The abdominal skin is cleansed with suitable agents e.g. Hibitane in alcohol and draped with a sterile towel. The site of entry is infiltrated down to the peritoneum with 1 percent Xylocaine using a 21G x $1\frac{1}{2}$" disposable needle. In many thin subjects liquor amnii can often be obtained via this needle and only the syringe need be changed to withdraw the required 10 mls. of liquor. If liquor is not obtained, particularly if the patient is obese, a disposable lumbar puncture needle (Brunswick 220 size 20 x $3\frac{1}{2}$") is inserted, liquor is withdrawn and placed in a sterile universal container which is immediately protected from light by wrapping in black paper or tinfoil.

The abdominal puncture, after removal of the needle, is sealed with Collodion and the patient taken to the canteen for a cup of tea. This enables her to be under observation for fifteen to twenty minutes and, if all is well, she goes home having been told to report immediately any adverse effect.

The specimen is centrifuged almost immediately at 3,000 rev./ minute for fifteen minutes. This is most important if blood-stained liquor has been obtained in order that the blood may be removed before haemolysis of the red cells takes place as this may mask the true bilirubin values. Centrifugation removes traces of vernix, epithelial cells etc. which may also be present.

The naked eye appearance of the supernatant is noted. Further examination is not carried out till the next day but the specimen is

protected from light and kept at 4-6°C. Protection from light is essential since bilirubin is decomposed rapidly on exposure to sunlight. Liley (1961) found that the pigment had a half life of ten hours in laboratory daylight and 12-18 minutes in winter sunshine. Protected from light he found no change in liquors examined after keeping in a refrigerator for nine months or after storage for thirty days at room temperature.

DANGERS OF AMNIOCENTESIS

Amniocentesis is not without danger to mother and child (Liley, 1960) but the value of the information obtained from liquor examination outweighs the risks.

The commonest hazards are as follows:-

(a) DANGER TO THE MOTHER

(1) INFECTION

A few cases of rigor and elevation of temperature which rapidly settled have been reported but generally infection is not a significant problem (Fairweather and Walker, 1964).

(2) ANTE-PARTUM HAEMORRHAGE

On occasion the placenta cannot be avoided particularly

where it is of the large type associated with Haemolytic Disease of the Newborn and lying anteriorly. If the needle puncture is clean there appears to be little risk of detachment of the placenta. If a vessel is pierced or damaged, blood may leak into the liquor and obscure the findings. Cases of abruptio placentae with risk to the mother and with fatal results to the foetus have been occasionally reported (Robertson, 1964).

(b) FOETAL DANGERS

(1) INFECTION

Infection to the foetus has rarely been reported (Robertson, 1964; Liley, 1960).

(2) DIRECT TRAUMA

In severely affected babies, enlarged by oedema, the needle may pierce the child. Unless a vital organ or foetal vessel is damaged, however, the foetus appears to come to little harm. Foetal exsanguination has been reported (Stenchever and Cibils, 1968). In this series in one patient the foetal heart could not be heard after blood-stained liquor had been obtained at amniocentesis and the ensuing intra-uterine death must be attributed to trauma of some kind. Post-mortem examination was inconclusive.

(3) FOETAL/MATERNAL BLEEDING

Foetal/Maternal bleeding may cause an increase in antibody level with additional foetal risk. This risk has been noted (Queenan and Adams, 1964; Peddle, 1968; Zipursky, Pollock, Chown and Israels, 1963) but reports by Freda (1965) and Fairweather, Murray, Parkin and Walker (1963) indicate that the risk is negligible and certainly should not contra-indicate amniocentesis.

In this series one case developed a saline antibody where only an antibody detectable by the indirect antiglobulin technique had been present before amniocentesis. This is said by Murray (1967) to carry a poor prognosis and this patient had a stillbirth. Her previous history, however, was poor. Two other patients had a rise in titre but this did not affect the outcome in so far as each had a live child which survived.

SOURCES OF ERROR IN EXAMINATION OF LIQUOR AMNII

(a) FLUIDS OTHER THAN LIQUOR AMNII

Such fluids may come from several sources but on close examination the nature of the fluid can be easily identified.

(1) MATERNAL URINE

This should rarely happen if the patient empties her bladder before amniocentesis but should such an accident

occur the fluid can be identified by smell, lack of vernix, lack of squamous cells and lanugo hairs.

(2) FOETAL ASCITIC FLUID

This is intensely pigmented fluid which can be recognised with the naked eye. Since this is obtained usually before or during intra-uterine transfusion the source can be recognised from the position of the needle as seen on an x-ray film.

Further error could be made by the aspiration of fluid from a meningocoele. This might be diagnosed by a good x-ray film and in any case examination of the protein constituents of this fluid and of ascitic fluid can establish the source with certainty. The protein constituents can be identified by electrophoretic techniques.

(3) MULTIPLE AMNIOTIC SACS

Plural pregnancy is diagnosed clinically. By the injection of Evans blue into one sac after amniocentesis the operator can make certain that further liquor is from the second sac which should be free from dye.

(4) AMNIOTIC CYSTS

These are rare. The fluid is turbid and has a high content of protein and fat which can be recognised by

biochemical analysis.

(b) PIGMENTS OTHER THAN BILIRUBIN

(1) SERUM AND HAEMOGLOBIN

Error is particularly likely if the liquor is contaminated with serum of an affected baby since this will contain high levels of bilirubin. The effect of such contamination is less if the baby is unaffected or if the serum is maternal for the reason that these contain much less bilirubin.

Where haemoglobin and red cells are contaminants a diagnosis of the source of the blood i.e. foetal or maternal can be made by alkali denaturation tests for haemoglobin and examination of the cells by grouping. Direct Anti Globulin test and a search for nucleated red cells. Haemoglobin contamination takes 2-3 weeks to clear from the liquor. Haemoglobin can be corrected for by extending the wavelengths over which spectrophotometric readings are made.

The type of curve obtained from liquor amnii contaminated by haemoglobin can be seen in Graph 1.

(2) MECONIUM

The presence of meconium is rare in the early part of the third trimester but in any case the absorption curve differs from that of bilirubin. Maximum absorption when

meconium is present is at 405-411 n. Heavy meconium-staining can cause difficulty in interpreting the result of liquor examination but it has been found that meconium clears from the liquor in a week and reliable readings are obtained once more. A graph illustrating this is shown on Page 82.

(3) CONGENITAL ABNORMALITIES

For some reason, perhaps related to the swallowing of liquor by the foetus, the bilirubin content of liquor is raised if congenital abnormality is present. In two non-sensitized cases examined, where hydramnios was present and liquor was examined out of interest, although no Rhesus problem was present, a high bilirubin content was found by spectrophotometry. One child had a meningomyelocoele and the other an exomphalos with associated duodenal atresia. Such an abnormality in a sensitized mother could well upset prediction results based on liquor examination.

(c) EFFECT OF MATURITY

(1) DOUBTFUL MENSTRUAL HISTORY

An error, in some cases, may be accounted for by a mistake in the patient's assessment of her expected date of confinement as the significance of the liquor reading varies with maturity.

(2) MATURITY AT DELIVERY

If a general assessment is made in terms of the infant's cord blood findings an apparent error may occur because of a planned or spontaneous premature delivery since, in premature deliveries, a higher cord blood haemoglobin may be found than in infants delivered at full term.

The early attempts to measure bilirubin and other elements in liquor amnii were discouraging (Beecham, Molthan, Boutwell and Rohrbeck, 1961; Watson, 1962) but this was probably in part due to the reluctance of clinicians to obtain adequate and frequent specimens of liquor amnii as this seemed to be an unjustifiable risk to mother and foetus. Recent work and, in particular, that of Liley (1961) has shown how useful the study of amniotic fluid is in the prediction of severity of Haemolytic Disease of the Newborn. It should be said, however, that each laboratory must develop its own methods and its own standards. The position has now been reached where no baby need be lost through inopportune intervention nor should a mother be allowed to deliver a severely affected child without the severity of the disease being known to her obstetrician, for it is now possible for the obstetrician to have a reasonably reliable assessment of the severity of the disease.

METHODS OF LIQUOR EXAMINATION

In 1961 Wild suggested that liquor bilirubin levels may be influenced by the protein level as had been demonstrated by Stempfel and Zetterstrom in 1955. Both values fall as pregnancy advances

so allowance must be made for the maturity of the foetus when interpreting results by these methods. Walker, Fairweather and Jones (1964) had calculated a bilirubin/protein ratio, measuring the protein by the Folin and Ciocalteau method and measuring the bilirubin spectrophotometrically. The findings of Walker et al (1964) were at variance with those of Cherry, Kochwa and Rosenfield (1965) and Morris, Murray and Ruthven (1967) who believed that bilirubin/protein ratio was of value in estimating the severity of Haemolytic Disease.

In 1969, Walker,Landon and Oxley repeated this work but using the Biuret method for protein estimation. They again found that the estimation of protein did not give as good a prediction of severity of Haemolytic Diseases as did bilirubin estimation alone and that there is nothing to be gained by linking bilirubin with protein estimations.

In 1967, Stewart, Taylor and Beck described a method by which bilirubin in amniotic fluid is estimated by a method in which bilirubin is coupled with Diazotized Sulfanilic acid in the presence of an aqueous accelerator and the subsequent Azobilirubin read photometrically. This is the same method which has been used for microplasma bilirubin estimations except that the sample size is increased forty times without changing the fluid sample volume. It is this fortyfold increase in sensitivity that enables the method to be used for the very low concentrations of bilirubin found in liquor amnii. These authors claim that by the use of this method more reliable results are obtained than by the use of spectrophotometry since the corrections for turbidity and/or haemoglobin

are uncertain in optical density methods. Such corrections are not, of course, necessary in the chemical methods of estimating bilirubin in liquor. Stewart et al successfully relate their predictions to the three zones of Liley as shown in Page 83 - mild, moderate and severe dependent on the bilirubin levels.

A further method of prediction by biochemical measurement of amniotic fluid bilirubin is suggested by Brazie, Bowes and Ibbott (1969). This consists of the extraction of the unconjugated bilirubin in liquor by shaking with chloroform and the determination of the bilirubin concentration in the chloroform layer. They claim that no interference with accuracy occurs in samples containing excessive haem pigments, meconium and turbidity and that the values obtained show excellent correlation with those obtained by the method of Liley for estimating the bilirubin by optical density at the 450n peak. These authors claim that their method is simple and is more sensitive and less cumbersome than standard methods of quantitating bilirubin by the Diazo reaction. They claim recoveries of bilirubin added to liquor amnii to be between 90-94 percent as do Pennington and Hall (1966) and that the coefficient of variation of estimation of bilirubin from amniotic fluid with chloroform was less than 1 percent at a level of 0.1 mgm./100 ml.

In their hands Brazie et al (1969) show that this method of bilirubin measurement is of value. The writer's experience suggests, however, that the extraction has to be repeated several

times and thus leads to inaccuracy from loss of bilirubin since the amount of bilirubin being measured is so small.

By far the most widely used methods of estimating the bilirubin content of liquor amnii are those involving spectrophotometry and the greatest contribution to this aid in prediction was made by Liley in 1961. Although Bevis (1956) had tried to relate kernicterus to liquor findings and Walker (1957) had used spectrophotometry to examine amniotic fluid and claimed 94.9% accuracy it was Liley who rationalised the method and his work has largely become the basic point for other workers in this field.

Liley (1961) showed that the absorption peak at 450n in the absorption curve increased in the presence of haemolytic anaemia but the significance of this value changed with maturity. He also showed the importance of the stage of pregnancy at which amniocentesis is done for the first time and emphasised the need for serial readings to show a rise or fall in maxima of the peak at 450n and thus obtained a trend which gives a better guide than one single reading. These results further suggested a correlation between the bilirubin peak at 450n and the cord haemoglobin level at delivery provided no more than one week interval elapsed between amniocentesis and delivery. In the construction of the absorption curve Liley made absorption readings at the following wavelengths:- 350, 365, 380, 390, 400, 410, 415, 420, 430, 440, 450, 460, 470, 485, 500, 515, 530, 540, 555, 570, 585, 600, 620, 640, 670 and 700n.

Liley also plotted the results on semilogarithmic graph paper with the optical densities as the ordinate on a logarithmic scale and the wavelength on the abscissa on a linear scale. He found

that the curve of normal liquor amnii was almost a straight line on such a graph. The wide range of wavelength at which measurements were made enabled distortion of the curve by the presence of oxyhaemoglobin, haemoglobin and methaemalbumen to be recognised. The optical density at 450n was measured from a base line drawn tangentially to the curve and joining the readings at 365 and 550n. Measuring from this base line eliminated the effects of contaminants such as haemoglobin and methaemalbumen on the peak at the 450n wavelength.

In a later paper Liley (1961) related bilirubin estimations to maturity and classified the results in a large series according to severity producing his classic prediction graph. Variations on this graph have been made by different workers but it remains the most frequently used reference in liquor examinations. This graph was constructed by plotting the observed difference in optical density (O.D.D.) on a logarithmic scale on the ordinate against weeks of maturity on the abscissa on a linear scale.

In assessing the results Liley found that the severely affected infants fell into the upper part of the graph, unaffected or mildly affected infants fitted into the lower part while a scatter of cases of varying severity from moderate to severe fell into the middle part and this group has proved to be the most difficult to assess with any accuracy.

He was thus able to divide the graph into three zones by constructing two parallel lines joining the points:-

(a) Optical Density (O.D.) 0.25 at 27 weeks' maturity with O.D. at 0.085 at 40 weeks

and

(b) O.D. 0.059 at 27 weeks' maturity with O.D. 0.02 at 40 weeks.

Following Liley's work many other workers have published results on the examination of liquor and its use in prediction of the severity of Haemolytic Disease.

Mackay (1961) presented a series of 223 patients and graded the liquor by visual inspection of graphs obtained by spectrophotometry and related these grades to the cord haemoglobin levels. He emphasised the fact that peaks representing pigments other than bilirubin existed at 410-415n (unidentified haempigments), 415, 540 and 575n (oxyhaemoglobin) and 630n (methaemalbumen) and that the presence of these in liquor amnii can affect the shape of the curve and perhaps mask and distort the peak at 450n. (This is illustrated in Graph 1 and V.). Mackay carried out amniocentesis initially at 28 weeks and later at 33 weeks.

There were many other studies carried out in the few years following Liley's papers (Walker, Fairweather and Knox, 1964; Alvey, 1964; Robertson, 1964).

One of the most useful studies was that of Freda (1965). He graded curves obtained from liquor amnii into four degrees of severity according to the optical density difference (O.D.D.) as follows:-

Grading of Severity	Difference of optical density values at 450n
1 +	0 - 0.2
2 +	0.2 - 0.35
3 +	0.35 - 0.7
4 +	0.7 or higher

The implications and actions which Freda suggested, based on his grades of severity were as follows:-

GRADE 1 +

Repeat amniocentesis at 10 day intervals for last 6 weeks of pregnancy.

GRADE 2 +

Repeat weekly amniocentesis. Baby Rhesus positive but no immediate danger.

GRADE 3 +

Baby Rhesus positive and some degree of circulatory failure present. If at 32 weeks' maturity deliver the patient.

GRADE 4 +

Impending foetal death usually within one week. No intervention of any value at this stage.

Freda at this time made the important point that the optical density difference height of the bilirubin peak (O.D.D.) was only an assessment of the foetus at that stage in its maturity.

Of the papers published in the late 1960's one of the most attractive is that of Whitfield, Neely and Telford (1968). In this study, which is based on the work of Liley, they suggest an "action line" superimposed on a Liley prediction graph which indicates, according to maturity, the necessity of immediate delivery or intra-uterine transfusion. The weakness of the method, however, is that it depends on the extrapolation of the trend of liquor findings derived from as little as two estimations. This is by no means as predictable as these authors suggest or alternatively it would involve the repetition of amniocentesis many more times. (This graph is shown on Page 84).

Since the procedure of amniocentesis has now been accepted as routine practice it is inevitable that the study of liquor amnii will become more detailed and further information will be obtained.

Another approach in the study of liquor amnii has been the estimation of Rhesus antibody titres in the liquor (Hoffbauer, 1967; Murray, 1969; Usategui-Gomez and Stearns, 1969; Usategui-Gomez, Stearns and Toolan, 1970). It would appear that this line of investigation will be a valuable adjunct to the conventional spectrophotometric examination of liquor.

Hoffbauer and Geburtsh (1967) simply related the presence of antibodies in the liquor amnii to the degree of severity of Haemolytic Disease of the babies delivered. He found a higher mortality and higher percentage of severely affected infants in cases where antibodies had been found in liquor. Murray (1969) reported a series of liquors in which Rhesus antibodies were found. She correlated the

titre of these liquor antibodies to that of the maternal serum using the indirect antiglobulin technique. This latter she said usually correlates to the severity of Haemolytic Disease, although exceptions do occur. Murray also pointed out the importance of using the sensitive "papainised cell" technique for the titration of antibodies in liquor amnii, to detect small amounts.

In 33 pregnancies Usategui-Gomez and Stearns (1969) showed that the maternal indirect antiglobulin antibody titre was related only to amniotic fluid titre only in so far as it determined the maximum found in the latter. In a later paper Usategui-Gomez, Stearns and Toolan (1969) found that there was great variation in the amount of antibody reaching the liquor amnii but they concluded that the Rhesus antibody titre of the amniotic fluid might be an indication of the effective level of antibody reaching the foetus. They further concluded that in their series where the amniotic fluid titre was 1/16 or more an affected child was delivered and in all cases where the titre was 1/32 or over the infants were all severely affected. They indicated that the level of antibody in the liquor amnii may be of use in the more accurate assessment of those cases which fall into the indeterminate zone 11 of Liley's prediction graph.

At present a study is being carried out in Glasgow and West of Scotland Regional Transfusion Centre investigating further the value of amniotic fluid antibodies with the additional parameter of quantitative antibody measurement using the auto-analysor technique. The series, while encouraging, is not yet large enough to give significant results (Mitchell, 1970).

Further benefits arising from the availability of amniotic fluid are the recent studies such as sex determination of the foetus from cells in the liquor amnii by Nelson and Emery (1969). This could be of great importance in the inheritance of sex-linked disorders where, for instance, a male foetus might have a serious sex-linked recessive disorder such as Duchenne muscular dystrophy. To the parents of such a foetus, termination of pregnancy can now be offered.

True estimation of maturity in a patient who is uncertain of her last menstrual period is often difficult. An attempt has been made by Anderson and Griffiths (1968) to estimate maturity by staining of cells present in liquor amnii which are thought to be from the foetal sebaceous glands and which appear in the liquor late in pregnancy. These contain fat droplets which stain orange with Nile Blue Sulphate. This technique, however, only appears to be of value in the last 4-6 weeks of pregnancy and is of little help in the mid-trimester and early third trimester when it can be difficult to make an accurate clinical estimation of maturity.

Amniography, the technique of injecting radio-opaque material into the amniotic sac and followed by x-ray examination, may give useful information on the stage of the foetus (Queenan, Von Gal and Kubarych, 1969). The displacement of the radio-opaque medium by the foetal soft tissues provides an excellent means of measuring the amount of foetal oedema. The features which can be evaluated are scalp oedema, ingestion of the radio-opaque medium, foetal attitude, protruberance of the abdomen and the scapular sign. The most important of these are scalp thickness, evidence of foetal swallowing and the scapular sign. Information about the volume

of amniotic fluid and size and situation of the placenta may also be obtained from the amniogram.

METHOD OF LIQUOR AMNII EXAMINATION USED IN THIS SERIES

A decision to perform amniocentesis on a patient is made on either or both of two findings:-

(1) An antibody level of 1/16 or greater by the indirect antiglobulin technique.

(2) A previous history of a severely affected baby.

If amniocentesis is carried out on antibody levels alone it is done at 29 and 31 weeks' gestation and repeated if necessary. Where there is a previous history of a severely affected baby, amniocentesis is carried out at 25 weeks' gestation and repeated at one or two weekly intervals as indicated. It may be necessary to make as many as 4 or 5 serial estimations at one or two weekly intervals so that the values obtained may be plotted on a Liley type prediction graph. Such results are useful in establishing whether the situation has changed and so indicates the trend of events. An occasional single late amniocentesis is carried out on patients who have been referred late i.e. at 33-35 weeks' gestation and who have no previous history available. As Liley (1961) found, however, these late single specimens can sometimes be misleading.

The specimens are obtained as described previously (Page 36) and centrifuged immediately in the hospital laboratory. The supernatant is recovered and placed in a sterile universal container protected from light. The spun deposit is similarly protected from

light and preserved for possible later examination e.g. blood grouping of red cells if present.

On arrival at the Regional Transfusion Centre Laboratory the specimen is again centrifuged and the supernatant recovered. If the liquor is cloudy it is filtered through a cellulose acetate filter using a small centrifugal filter unit. Asbestos filter pads are avoided because it was found that there was some loss of bilirubin by absorption in the asbestos pad. (Graph V1 - Page 86).

SPECTROSCOPIC EXAMINATION

The liquor is scanned on a Unicam S.P. 500 spectrophotometer over the range of wavelength 350n - 700n. Readings are taken at 5n intervals up to 600n and thereafter at 20n intervals unless the liquor has a brown tinge and the presence of methaemalbumen is suspected. If methaemalbumen is suspected the liquor is scanned at 5n intervals to 640n and thereafter at 20n intervals to 700n.

A graph is drawn on semi-logarithmic graph paper with the optical density on a logarithmic scale on the ordinate and the wavelength on a linear scale on the abscissa. A base line is drawn joining the points on the curve at 600n and 520n and this straight line is projected back to cut the 450n ordinate. The difference in optical density between the point at which this line cuts the 450n ordinate to the point on the curve at 450n is measured. This is rather loosely called the height at 450n or optical density difference (O.D.D.) and is used as a measure of the concentration of bilirubin in the liquor. (This is illustrated on Graph V11).

As previously stated (Page 49) the presence of haemolgobin (absorption maxima 415n, 540n, 575n) causes distortion of the curve. Using Liley's method for determination of O.D.D. (Page 47) it was found that chemical findings in the first series of 50 cases did not fit well on Liley's prediction graph and this was attributed to the effects of contamination of the liquor with haemoglobin pigments.

A series of artificial mixtures of bilirubin and haemoglobin were examined by spectrophotometer and from these experiments the base line drawn as described above appeared to give recovery results for added bilirubin with greater accuracy than the original Liley line and in addition there was a better correlation with the clinical findings in the 50 cases then under review. (Graphs 1 and V111).

CLASSIFICATION

Data for O.D.D. for 50 cases were plotted as ordinates on a semi-logarithmic scale against weeks of maturity on a linear scale as abscissa and graded for severity by the use of the three zones as described by Liley.

In this series the correlation of these observations to clinical assessment was not as good as expected. A better correlation was obtained by drawing the two parallel lines at the following positions:-

(1) Upper line from optical density at 0.2 at 26 weeks to optical density 0.09 at 40 weeks.

(2) Lower line from optical density at 0.078 at 26 weeks to optical density 0.034 at 40 weeks.

If the liquor samples are taken earlier than 26 weeks these lines can be projected back to the ordinate axis, parallel to the abscissa.

As used by Liley the O.D.D. at 450n which lie above the upper line indicate a severely affected baby and the possibility of intra-uterine transfusion or early delivery must be considered. Those in the middle zone are considered to be moderately affected - perhaps requiring induction at 37 or 38 weeks with the possibility of an exchange transfusion being necessary - but not in danger of intra-uterine death. Those below the lower line are considered to be mildly affected or unaffected.

As previously stated, where serial determinations were made, close attention is paid to the trend of the readings as well as to the actual O.D.D. values.

FURTHER OBSERVATIONS ON LIQUOR SPECIMENS

Various workers, Fleming, Wolffe, Murray (1965); Bjerre, Gold, Wilson, Doran (1968) and Connon (1969) have proposed formulae from which the concentration of bilirubin in liquor can be calculated in mgm.% by the use of spectrophotometry. These formulae usually include factors to correct for the effect of haemoglobin, met-haemalbumen and turbidity. Results using such formulae are sometimes referred to as "Calculated Bilirubin".

The formula suggested by Fleming, Wolffe and Murray (1965) is as follows:-

$$1.25 \times (O.D.460) - 0.91 \times (O.D.576) - 1.15 \times (O.D.623) + 0.11 \times (O.D.700)$$

Factor for bilirubin	Factor for haemolgobin	Factor for methaemalbumen	Factor for turbidity

This formula gives the bilirubin level in milligrammes percent and as with the O.D.D. at 450n the results can be classified into zones.

This calculation is done routinely on all specimens of liquor as an additional check. The total protein is also estimated by the Biuret method but this is of limited value.

Recently, as suggested by Murray (1969); Usategui-Gomez, Stearns and Toolan (1969), the remainder of the specimen is being examined for the presence of Rhesus antibodies and the anti D content estimated in microgrammes by the automated technique. To date there is insufficient data available for discussion of the value of this parameter.

RESULTS

The introduction of amniocentesis and the study of liquor

amnii has produced an invaluable tool for the antenatal prediction of Haemolytic Disease of the Newborn. Initially the liquor findings were correlated with the clinical assessment based on Liley's work. Results showed a 17% error. A number of predictions by O.D.D. and by clinical results did not agree in certain cases classified as intermediate.

The main difficulty arose in classification of the baby itself. Classification of the baby's clinical condition is largely subjective. In addition the levels of haemoglobin and bilirubin in cord blood used for the classification of mild, moderate and severely affected babies vary a great deal from worker to worker (Liley, 1961; Queenan, 1969; Robertson, 1969; Walker, Fairweather and Jones, 1964).

If classification by treatment of the baby was used then in this series the situation was even more complicated by a change in Paediatric policy which took place during the course of this investigation. In the early part of the series, exchange transfusion was carried out soon after birth on a fixed basis of cord haemoglobin of 12 gm.% or less and/or a cord bilirubin of 3.5 mgm.% or more. Thereafter a policy of "wait and see" was adopted, based on withholding exchange transfusion if the cord haemoglobin was 10 gm.% or over and/or cord bilirubin was 5 mgm.% or less. Exchange transfusion was started only when the haemoglobin fell below 10 gm.% or bilirubin rose to 18-20 mgm.%. Several of these babies treated by the "wait and see" method required 2 - 3 exchange transfusions because of developing anaemia and/or hyperbilirubinaemia. Such an infant could be classed as severely affected, when on the

basis of cord blood findings at birth, it would be classed as mild or moderate.

Classification or assessment is made difficult because of the many variables which exist e.g. the maturity of the baby and hence its ability to cope with the haemolysis of red cells and hyperbilirubinaemia, activity of antibody present in the baby's blood, activity of the haemopoetic tissue etc.

Looking at the picture as a whole it was concluded that in fact three stages capable of assessment are present in Haemolytic Disease - the antenatal stage as judged by liquor amnii examination; the immediate stage at birth judged by clinical examination and cord blood levels of haemoglobin and bilirubin of which haemoglobin is the more important, and the later postnatal stage of hyperbilirubinaemia with the danger of kernicterus. This last stage cannot be predicted accurately from liquor amnii examination.

THE CORD BLOOD FACTOR

It was appreciated that many severely affected babies with a low cord haemoglobin often have relatively low cord bilirubin. Bilirubin is a product of red cell haemolysis and antenatally excess bilirubin is cleared by the placenta. This may account for some low bilirubin levels at birth. If it is accepted that the level of haemoglobin at birth is a reflection of the rate of breakdown of haemoglobin it is possible that there is, in fact, some real relationship between cord haemoglobin and cord bilirubin at birth.

It was decided, therefore, to calculate for the cases under review the ratio cord haemoglobin (gm.%)/cord bilirubin (mgm.%). For convenience this figure has been called the Cord Blood Factor (C.B.F.).

Table 1 shows the cord blood findings and C.B.F. for the series of 173 cases. It is felt that this parameter gives a useful indication of the conditions existing inthe infant's blood at birth and appears to correlate well with the liquor findings when the C.B.F. is divided into mild, moderate and severe sections as is the O.D.D. of the liquor findings.

The C.B.F. values corresponding to liquor mild, moderate and severe divisions are as follows:-

Mild	-	Over 3
Moderate	-	1 - 3
Severe	-	Under 1

The haemoglobin values for the baby are taken as follows:-

Mild	-	Over 12 gm.
Moderate	-	10 - 12 gm.
Severe	-	Under 10 gm.

The bilirubin values for the baby are as follows:-

Mild	-	3.5 mgm. or less
Moderate	-	3.5 mgm. - 5 mgm.
Severe	-	Over 5 mgm.

"Over" prediction was said to occur if the liquor prediction was more severe than the baby judged by the above parameters and "under" prediction if it was less severe than the baby judged by these parameters.

TABLE 1

Baby No.	Cord Haem.	Cord Bil.	Cord Factor	Liquor Prediction
1	16.2	3.2	5.1	Mild
2	9.0	4.2	2.1	Mod.
3	16.0	3.1	5.2	Mild
4	18.0	2.3	7.8	Mild
5	17.0	4.0	4.3	Mod
6	14.4	6.9	2.1	Mod
7	15.7	6.1	2.6	Mild
8	7.2	4.3	1.7	Mod.
9	16.2	3.6	4.5	Mild
10	13.6	2.5	5.4	Mild
11	13.6	1.4	9.7	Mild
12	6.2	5.8	1.1	Severe
13	11.0	5.3	2.1	Mod.
14	13.6	1.2	11.3	Mild
15	19.6	1.9	10.3	Mild
16	6.3	6.2	1.0	Mild
17	9.8	4.5	2.2	Mod.
18	12.0	4.5	2.7	Mod.
19	15.0	2.7	5.6	Mild
20	13.7	2.0	6.9	Mild
21	9.2	2.0	4.6	Mild
22	13.6	1.9	7.2	Mild
23	4.0	6.2	0.65	Severe
24	16.2	2.6	6.2	Mild
25	10.5	2.6	4.0	Mild
26	6.7	3.9	1.7	Mod.
27	5.6	7.9	0.6	Severe
28	14.3	3.7	3.9	Mild
29	14.0	2.7	5.2	Mild
30	9.6	6.2	1.5	Mild

TABLE 1 (continued)

Baby No.	Cord Haem.	Cord Bil.	Cord Factor	Liquor Prediction
31	11.8	2.0	5.9	Mild
32	16.4	2.0	8.2	Mild
33	10.3	7.2	1.4	Mod.
34	10.5	4.2	2.5	Mild
35	14.3	5.5	2.7	Mod.
36	10.7	5.6	1.9	Mod.
37	12.5	3.7	3.4	Mild
38	18.0	2.3	7.8	Mild
39	15.6	2.5	6.2	Mild
40	13.8	3.6	3.8	Mild
41	9.7	3.7	2.6	Mild
42	15.0	1.9	8.0	Mild
43	11.5	2.1	5.5	Mod.
44	11.0	2.2	5.0	Mild
45	16.0	2.1	7.6	Mild
46	15.8	0.8	19.8	Mild
47	16.0	1.5	10.7	Mild
48	9.0	6.0	1.5	Mod.
49	11.2	3.2	3.5	Mild
50	8.9	6.2	1.4	Mod.
51	20.0	4.3	4.7	Mild
52	5.3	5.9	0.9	Mild
53	13.8	2.1	6.6	Mild
54	14.0	3.2	4.4	Mild
55	13.0	3.4	3.8	Mild
56	14.0	2.8	5.7	Mild
57	14.4	4.4	3.3	Mild
58	12.6	2.8	4.3	Mild
59	15.8	2.1	7.5	Mild
60	14.6	3.6	4.1	Mild

TABLE 1 (continued)

Baby No.	Cord Haem.	Cord Bil.	Cord Factor	Liquor Prediction
61	14.8	2.2	6.7	Mild
62	16.8	2.5	6.7	Mild
63	17.6	2.5	7.0	Mild
64	10.5	5.0	2.1	Mild
65	12.6	2.6	4.8	Mild
66	13.4	3.4	3.9	Mild
67	13.6	2.6	5.2	Mild
68	15.4	2.8	5.5	Mild
69	20.0	1.2	16.7	Mild
70	17.5	2.9	6.0	Mild
71	11.5	2.9	4.0	Mod.
72	17.0	1.7	10.0	Mild
73	18.4	2.5	7.4	Mild
74	6.8	5.6	1.2	Severe
75	15.0	1.7	8.8	Mild
76	13.2	4.8	2.8	Mod.
77	16.2	1.5	10.8	Mild
78	17.8	1.8	10.0	Mild
79	9.0	5.0	1.8	Mod.
80	13.9	3.9	3.5	Mild
81	17.0	3.6	4.7	Mild
82	11.6	3.6	3.2	Mod.
83	9.7	4.2	2.3	Mod.
84	12.8	3.4	3.8	Mild
85	13.0	2.0	6.5	Mild
86	18.0	1.6	11.3	Mild
87	13.0	2.8	4.6	Mild
88	5.5	6.3	0.9	Mod.
89	14.6	1.8	8.1	Mild
90	10.0	8.7	1.2	Mod.

TABLE 1 (continued)

Baby No.	Cord Haem.	Cord Bil.	Cord Factor	Liquor Prediction
91	10.0	5.1	2.0	Mild
92	10.7	5.3	2.0	Mild
93	16.0	3.3	4.9	Mod.
94	14.8	1.5	9.9	Mild
95	13.2	8.3	1.6	Mod.
96	14.0	6.3	2.2	Mod.
97	16.6	2.1	7.9	Mild
98	16.0	4.0	4.0	Mild
99	12.1	3.0	4.3	Mild
100	14.0	1.9	7.4	Mild
101	15.7	2.6	4.4	Mild
102	2.3	3.6	0.64	Severe
103	14.4	2.5	5.7	Mild
104	11.2	3.4	3.3	Mild
105	14.8	2.8	5.3	Mild
106	9.4	9.3	1.0	Mod.
107	12.8	3.8	3.4	Mild
108	10.8	4.3	2.1	Mod.
109	12.0	4.7	2.6	Mod.
110	13.4	2.4	5.5	Mild
111	10.7	5.1	2.1	Mod.
112	15.2	1.8	8.5	Mild
113	18.6	2.7	6.9	Mild
114	8.4	8.6	0.9	Mod.
115	11.6	4.3	2.7	Mild
116	7.7	6.7	1.1	Mod.
117	10.2	8.7	1.0	Mod.
118	10.4	1.2	1.2	Mod.
119	15.8	2.3	6.9	Mod.
120	19.3	2.8	6.9	Mild

TABLE 1 (continued)

Baby No.	Cord Haem.	Cord Bil.	Cord Factor	Liquor Prediction
121	2.5	3.6	0.69	Severe
122	16.8	2.6	6.5	Mild
123	5.5	6.7	0.82	Severe
124	6.1	6.8	0.89	Severe
125	14.0	3.0	4.7	Mild
126	12.0	3.5	3.4	Mild
127	18.0	4.2	4.3	Mod.
128	18.0	2.0	9.0	Mild
129	18.8	3.0	6.3	Mild
130	17.9	2.6	6.9	Mild
131	18.0	3.8	4.7	Mild
132	19.6	1.5	13.0	Mild
133	13.4	2.8	4.8	Mild
134	10.2	6.9	1.5	Mod.
135	13.8	4.0	3.5	Mild
136	3.2	5.2	0.61	Severe
137	14.5	3.6	4.0	Mild
138	14.0	3.7	3.8	Mild
139	16.7	2.0	8.4	Mild
140	14.8	4.9	3.0	Mild
141	15.1	2.5	6.0	Mild
142	10.2	4.5	2.3	Mod.
143	15.7	2.5	6.3	Mild
144	13.3	4.1	3.2	Mild
145	16.8	2.0	8.4	Mild
146	11.1	6.2	1.8	Mod.
147	16.0	2.5	6.4	Mod.
148	8.6	2.7	3.2	Mild
149	13.2	3.8	3.5	Mild
150	13.0	4.4	2.9	Mild

TABLE 1 (continued)

Baby No.	Cord Haem.	Cord Bil.	Cord Factor	Liquor Prediction
151	14.0	2.8	5.0	Mild
152	12.0	4.5	2.7	Mild
153	14.3	2.3	6.2	Mild
154	13.8	2.5	5.5	Mild
155	18.0	2.3	7.8	Mild
156	16.3	1.5	9.9	Mild
157	14.5	6.4	2.3	Mild
158	18.0	1.8	10.0	Mild
159	17.3	1.5	11.6	Mild
160	9.8	5.3	1.8	Mod.
161	20.0	1.8	16.4	Mild
162	19.3	2.7	7.1	Mild
163	14.8	3.0	4.9	Mild
164	12.0	4.9	2.4	Mild
165	9.1	7.1	1.3	Mild
166	16.0	2.3	6.9	Mild
167	12.5	3.0	4.2	Mild
168	13.6	1.8	7.5	Mild
169	14.3	2.0	7.2	Mild
170	14.0	2.9	4.8	Mild
171	3.0	4.2	0.7	Severe
172	18.0	2.0	9.0	Mild
173	19.8	2.0	9.0	Mild

Table No. 11 shows the cord blood findings in relation to liquor prediction in the 173 cases. In addition it shows the range of cord haemoglobin, cord bilirubin and cord factor, and indicates the percentage of cases in each range. The mean and the standard deviations are shown. These findings are further illustrated in the column diagrams on Pages 71, 72, 73 and 74.

TABLE 11

CORD BLOOD FINDINGS IN RELATION TO LIQUOR PREDICTIONS IN 173 CASES

CORD HB. (gms.%)				CORD BILIRUBIN (mgm.%)				CORD FACTOR			
	Liquor				Liquor				Liquor		
Range	Mild	Mod.	Sev.	Range	Mild	Mod.	Sev.	Range	Mild	Mod.	Sev.
0-0.9	-	-	-	0 -0.4	-	-	-	0 -0.4	-	-	-
1-1.9	-	-	-	0.5-0.9	1	-	-	0.5-0.9	1	2	8
2-2.9	-	-	2	1.0-1.4	3	1	-	1.0-1.4	2	7	2
3-3.9	-	-	2	1.5-1.9	19	-	-	1.5-1.9	1	9	-
4-4.9	-	-	1	2.0-2.4	23	2	-	2.0-2.4	6	9	-
5-5.9	1	1	2	2.5-2.9	31	2	-	2.5-2.9	5	4	-
6-6.9	1	1	3	3.0-3.4	13	1	-	3.0-3.4	8	1	-
7-7.9	-	2	-	3.5-3.9	14	2	2	3.5-3.9	10	-	-
8-8.9	1	2	-	4.0-4.4	8	6	1	4.0-4.4	9	3	-
9-9.9	4	7	-	4.5-4.9	3	5	-	4.5-4.9	11	1	-
10-10.9	5	9	-	5.0-5.4	3	3	1	5.0-5.4	9	-	-
11-11.9	5	5	-	5.5-5.9	1	3	2	5.5-5.9	6	1	-
12-12.9	10	2	-	6.0-6.4	4	4	1	6.0-6.4	7	1	-
13-13.9	21	2	-	6.5-6.9	-	3	2	6.5-6.9	10	1	-
14-14.9	22	3	-	7.0-7.4	1	2	-	7.0-7.4	6	-	-
15-15.9	12	1	-	7.5-7.9	-	-	1	7.5-7.9	7	-	-
16-16.9	16	2	-	8.0-8.4	-	1	-	8.0-8.4	5	-	-
17-17.9	7	1	-	8.5-8.9	-	3	-	8.5-8.9	2	-	-
18-18.9	11	1	-	9.0-9.4	-	1	-	9.0-9.4	3	-	-
19-19.9	5	-	-	9.5-9.9	-	-	-	9.5-9.9	3	-	-
20-20.9	3	-	-	10.0-10.4	-	-	-	10.4-10.4	4	-	-
21-21.9	-	-	-					10.5-10.9	2	-	-
								11.0-11.4	2	-	-
								11.5-11.9	1	-	-
								12.0-12.4	-	-	-
								12.5-12.9	-	-	-
								13.0-13.4	1	-	-
								16.0-16.4	1	-	-
								16.5-16.9	1	-	-
								19.5-20.0	1	-	-
TOTALS	124	39	10	TOTALS	124	39	10	TOTALS	124	39	10
MEAN	14.6	11.2	4.5	MEAN	2.9	5.2	5.6	MEAN	5.0	2.4	0.8
S.D.	2.8	2.8	1.6	S.D.	1.2	1.9	1.4	S.D.	3.1	1.5	0.2
RANGE (MEAN ± 2 S.D.)				RANGE (MEAN ± 2 S.D.)				RANGE (MEAN ± 2 S.D.)			
MILD	9.0 - 20.2			MILD	0.5 - 5.3			MILD	0 - 12.2		
MOD.	5.6 - 16.8			MOD.	1.4 - 9.0			MOD.	0 - 5.4		
SEV.	1.3 - 7.7			SEV.	2.8 - 8.4			SEV.	0.4 - 1.2		

Figures 1, 11 and 111 are diagrams which show the frequency distribution of the 173 cases of liquor prediction related to cord haemoglobin gms. percent, cord bilirubin mgm. percent and cord blood factor respectively.

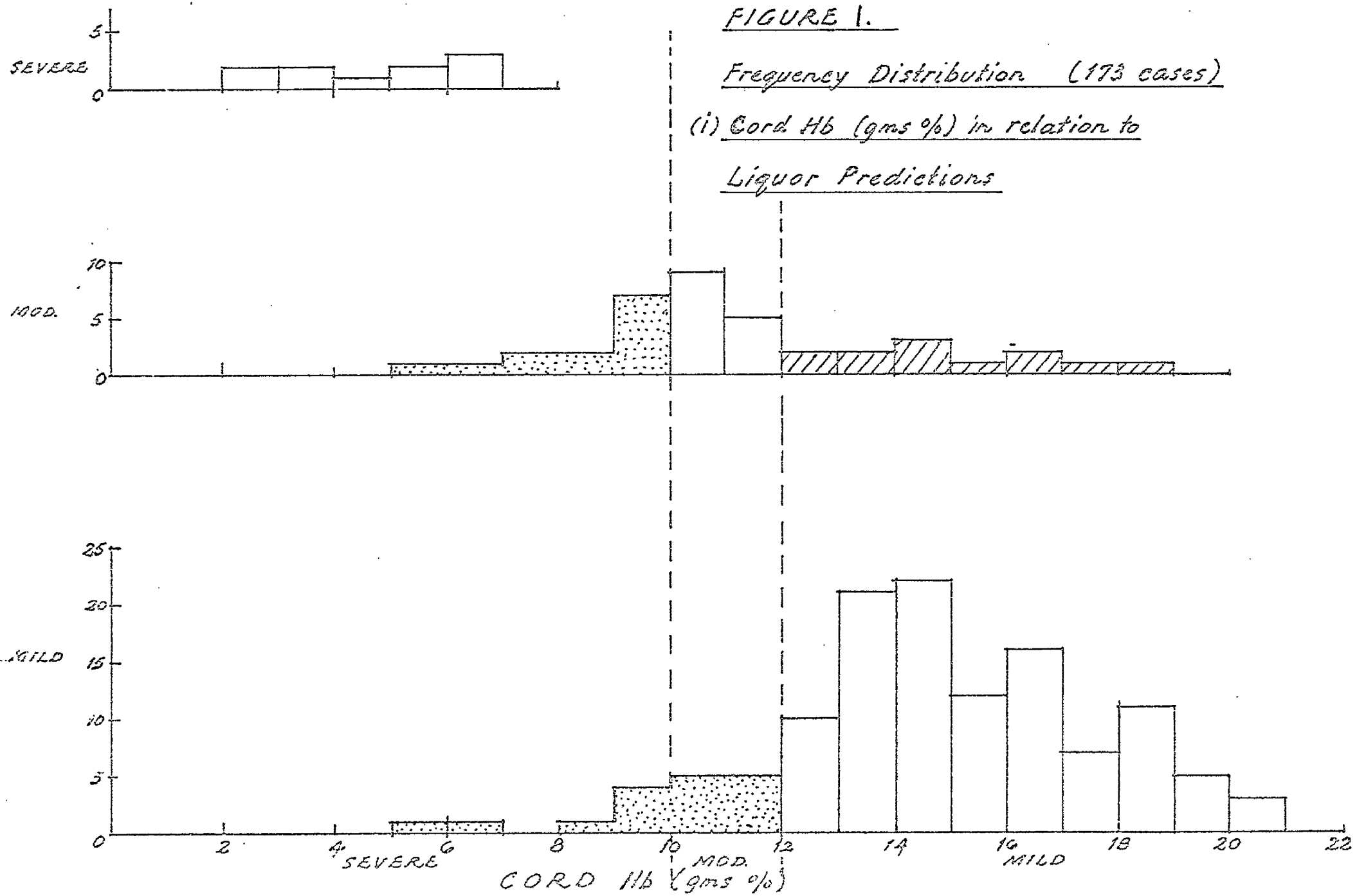
The key to these figures is to be found on Figure 111.

Figure 1V is a diagrammatic illustration of the cord blood findings in relation to liquor prediction showing the mean ± 1 and 2 standard deviations.

FIGURE 1.

Frequency Distribution (173 cases)

(i) Cord Hb (gms %) in relation to Liquor Predictions



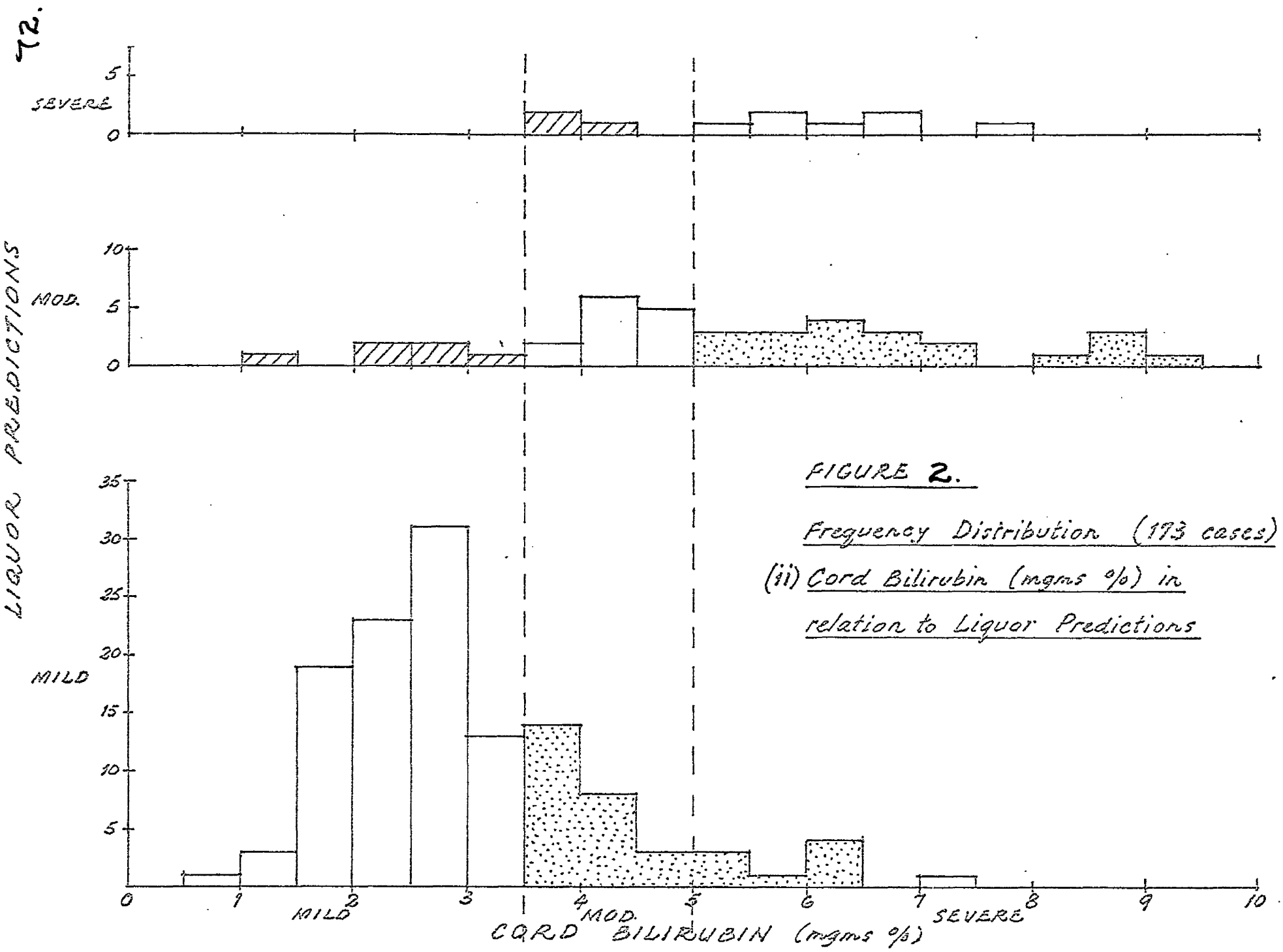


FIGURE 2.

Frequency Distribution (173 cases)

(ii) Cord Bilirubin (mgms %) in relation to Liquor Predictions

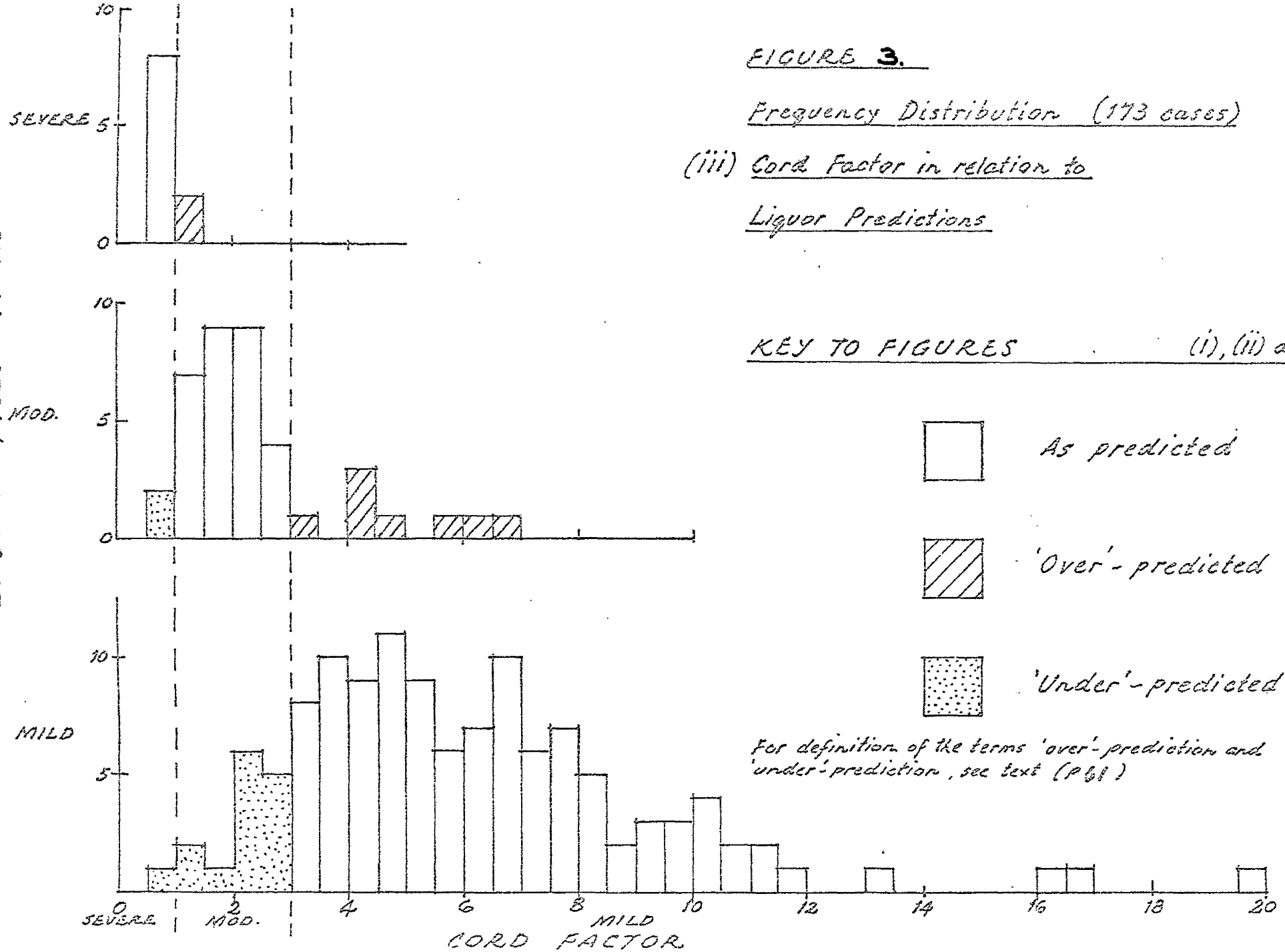


FIGURE 3.

Frequency Distribution (173 cases)

(iii) Cord Factor in relation to Liquor Predictions

KEY TO FIGURES (i), (ii) and (iii)

As predicted

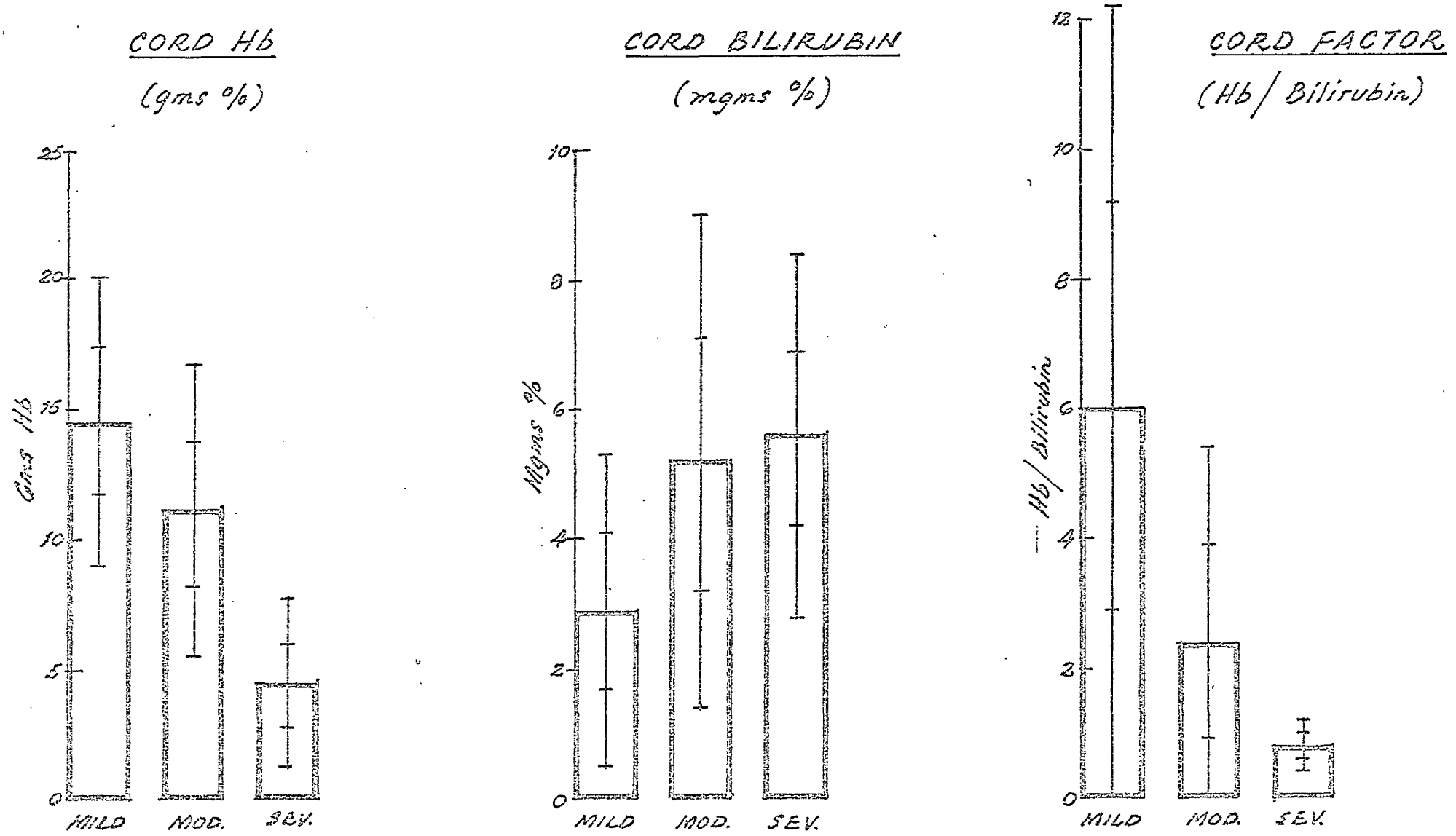
'Over'-predicted

'Under'-predicted

For definition of the terms 'over'-prediction and 'under'-prediction, see text (p61)

FIGURE 4. Cord Blood Findings in Relation to Liequor Prediction

Graphic illustration of Mean ± 1 SD and Mean ± 2 SD



LIQUOR PREDICTIONS

Since Liley in 1961 related liquor amnii bilirubin to maturity in his prediction graph this has been the most reliable parameter in the antenatal assessment of the severity of Rhesus isoimmunization as affecting the baby. The assessment of the severity of Haemolytic Disease at birth has been a most difficult problem and Liley found the haemoglobin status of the baby at birth correlated best with his liquor prediction. This, however, gave a 17% error. In this series liquor amnii predictions have been correlated with cord haemoglobin, cord bilirubin and a new cord blood factor. The errors in prediction in the series as seen in Table 111 have been as follows:- 23.1% correlated with haemoglobin; 36.4% correlated with bilirubin and 15.6% correlated with cord blood factor.

From the above figures it is confirmed that liquor amnii examination is of great value in prediction of severity of Haemolytic Disease of the Newborn. It is suggested cord blood factor correlates best with that prediction and appears to be a useful parameter in the assessment of the baby at birth.

TABLE 111

ERRORS IN LIQUOR PREDICTION RELATED TO CORD BLOOD FINDINGS IN 173 CASES

Liquor Prediction*	Liquor Prediction/ Cord Blood Findings	Cord Hb.	Cord Bilirubin	Cord Factor
"UNDER"	Mild/Moderate	10 (5.8%)	25 (14.5%)	14 (8.1%)
	Moderate/Severe	13 (7.5%)	20 (11.6%)	2 (1.2%)
	Mild/Severe	7 (4.0%)	9 (5.2%)	1 (0.6%)
	TOTAL	30 (17.3%)	54 (31.2%)	17 (9.8%)
"OVER"	Severe/Moderate	None	3 (1.7%)	2 (1.2%)
	Moderate/Mild	10 (5.8%)	6 (3.5%)	8 (4.6%)
	Severe/Mild	None	None	None
	TOTAL	10 (5.8%)	9 (5.2%)	10 (5.8%)
ERRORS	TOTALS	40 (23.1%)	63 (36.4%)	27 (15.6%)

* For definitions of "under" and "over" liquor predictions, see text. (Page 61).

STILLBIRTHS AND NEONATAL DEATHS IN THE AMNIOCENTESIS CASES

(See Table 1V).

In the 192 cases of amniocentesis there were 15 stillbirths (7.8%) and 11 neonatal deaths (5.7%). The 192 cases are the more severely affected cases out of a total of 917 excluding the 26 cases treated by intra-uterine transfusion which are dealt with separately. The results are shown in Table 1V.

Of the stillbirths, 10 had hydrops foetalis and in all these cases the liquor prediction was severe. Three cases had an unexplained intra-uterine death. The liquor prediction in these was mild. In 2 cases with a moderate prediction the cause of death was antepartum haemorrhage.

Of the neonatal deaths, 8 babies had hydrops foetalis and all of these had severe liquor predictions. Two babies died of Respiratory Distress Syndrome. One of these had a mild liquor prediction and did not require treatment for Haemolytic Disease. The other had a severe liquor prediction and had two exchange transfusions. One baby which had a moderate liquor prediction died unexpectedly during exchange transfusion.

There are 19 more cases discussed above than in the part dealing with cord blood findings because in these 19 cases cord blood examination was not possible.

TABLE IV

Stillbirths and Neonatal Deaths in the Amniocentesis Cases

No.	Cause	Liq.	Hb.	Bil.	C.B.F.	S.B.	N.N.I
1	Hydrops	Severe	Severe	M & S	Mod.	-	1
2	Hydrops	Severe	Severe	M & S	S	1	-
3	Exchange death	Mod.	Mod.	M & S	Mod.	-	1
4	Hydrops	Severe	Severe	M & S	Mod.	-	1
5	Hydrops	Severe	Severe	Mild	S	-	1
6	Hydrops	Severe	Severe	Mild	S	-	1
7	Hydrops	Severe	N.D.	-	-	1	-
8	Hydrops	Severe	N.D.	-	-	1	-
9	Hydrops	Severe	-	-	-	1	-
10	Hydrops	Severe	-	-	-	1	-
11	Hydrops	Severe	Severe	M & S	S	-	1
12	Hydrops	Severe	-	-	-	1	-
13	Hydrops	Severe	-	-	-	1	-
14	A.P.H.	Mod.	-	-	-	1	-
15	Hydrops	Severe	-	-	-	-	1
16	R.D.S.	Mild	Mod.	M & S	Mod.	-	1
17	Hydrops	Severe	-	-	-	1	-
18	Hydrops	Severe	Severe	M & S	S	-	1
19	A.P.H.	Mod.	-	-	-	1	-
20	I.U.D. unexplained	Mild	-	-	-	1	-
21	Hydrops	Severe	-	-	-	1	-
22	I.U.D. in labour unexplained	Mild	-	-	-	1	-
23	I.U.D. unexplained	Mild	-	-	-	1	-
24	Hydrops	Severe	-	-	-	1	-
25	2 exchange R.D.S.	Severe	Severe	M & S	S	-	1
26	Hydrops	Severe	Severe	Mild	S	-	1

The key to this table is overleaf.

KEY:

R.D.S.	-	Respiratory Distress Syndrome
M & S	-	Moderate and Severe
I.U.D.	-	Intra-uterine death
C.B.F.	-	Cord Blood Factor
A.P.H.	-	Ante-partum haemorrhage
N.N.D.	-	Neonatal death
S.B.	-	Stillbirth
N.D.	-	Not done
Mod.	-	Moderate
Hb.	-	Haemoglobin
Liq.	-	Liquor amnii

SUMMARY

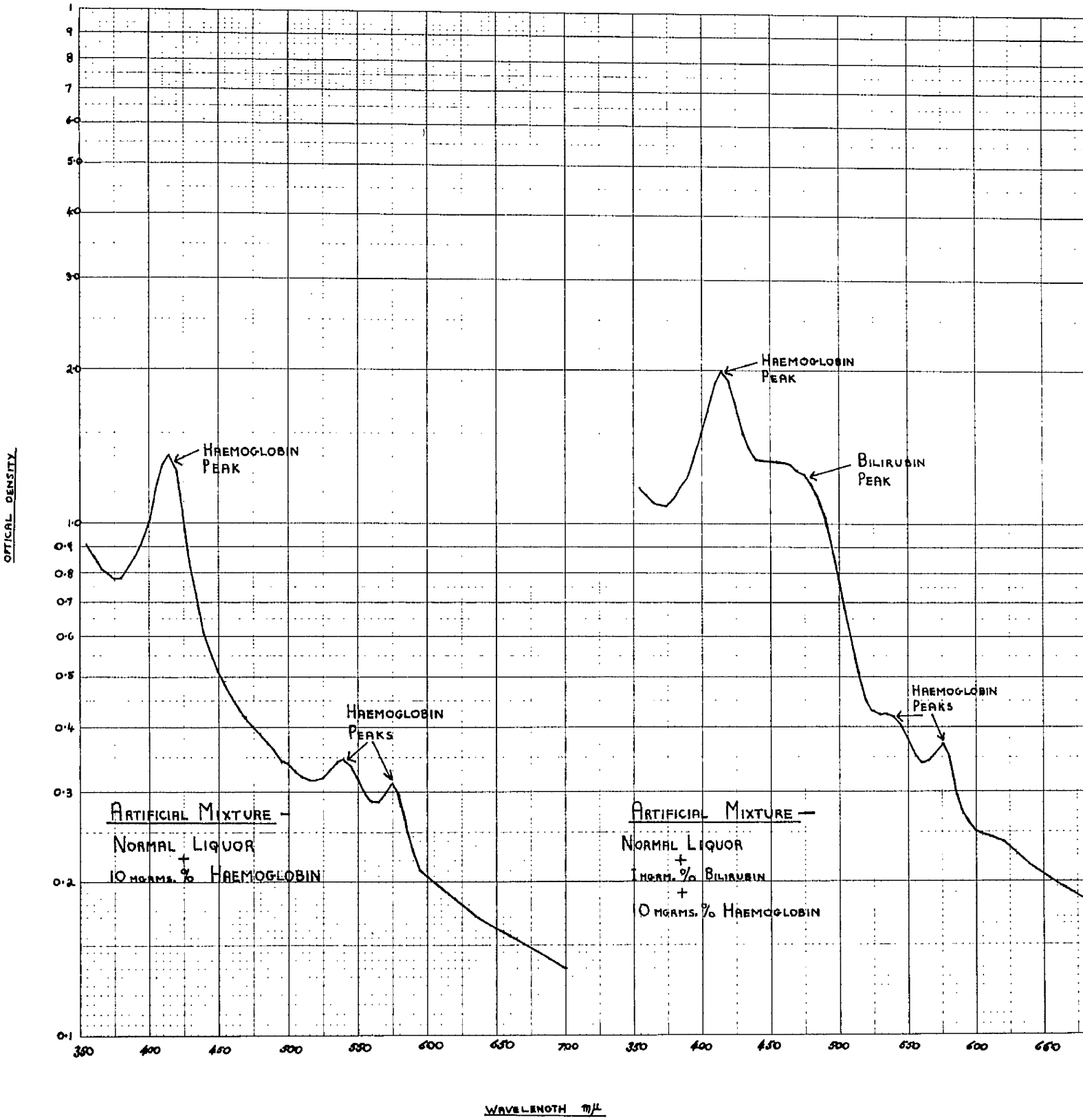
The study of liquor amnii has now become firmly established in the management of the Rhesus isoimmunized pregnant woman. It should not, however, be used indiscriminately. It does carry a slight, but definite, risk to mother and foetus and does not entirely replace the conventional standards of prediction of the degree of severity as affecting the baby. The most rewarding use of liquor examination is to detect the severely affected baby and the mild or unaffected baby. In the former case action can be planned accordingly and the baby given the best possible chance of survival. In the latter case accurate prediction can prevent interference, with a potentially disastrous result, in a Rhesus negative baby. It is fortunate that liquor examinations, by whatever method - spectrophotometric or biochemical, are most reliable in these two extremes.

Where the prediction by liquor examination lies in the middle zone the picture is less accurate, but much additional help can be obtained by using the longer established methods of prediction - maternal antibody titres and now antibody content, previous history or pattern of disease and zygosity of the husband. There is no doubt, however, that liquor amnii examination is the best single diagnostic aid provided its limitations are noted, particularly that it reveals the state of the baby only at the time of amniocentesis.

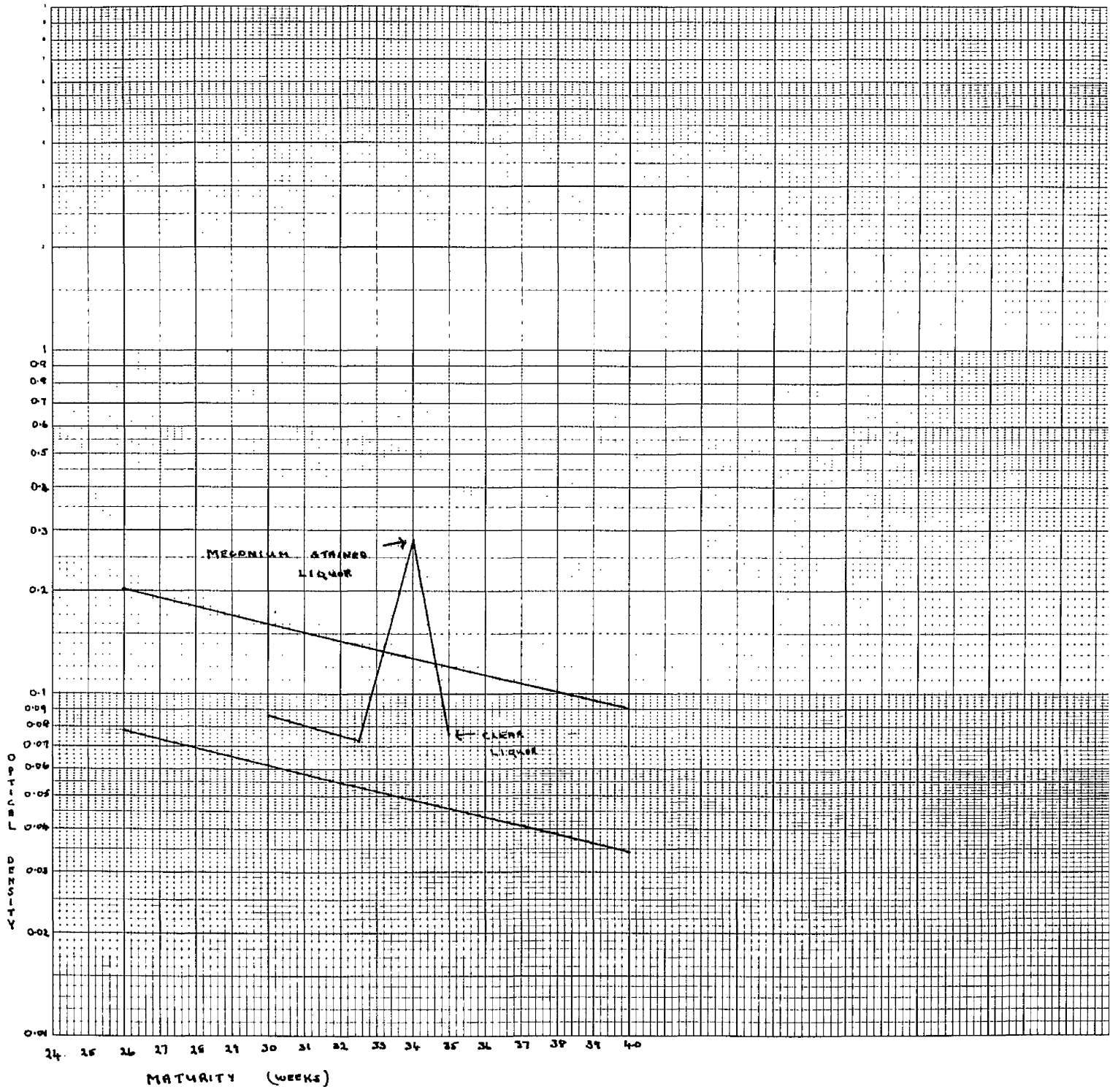
It is true to say that even with amniocentesis, and the other aids to prognosis, the eventual survival of many affected babies depends on intelligent interpretation of these parameters and previous experience.

In the present series it was found that liquor amnii examination was a most valuable prognostic aid for the antenatal management of Rhesus sensitized patients.

GRAPH 1

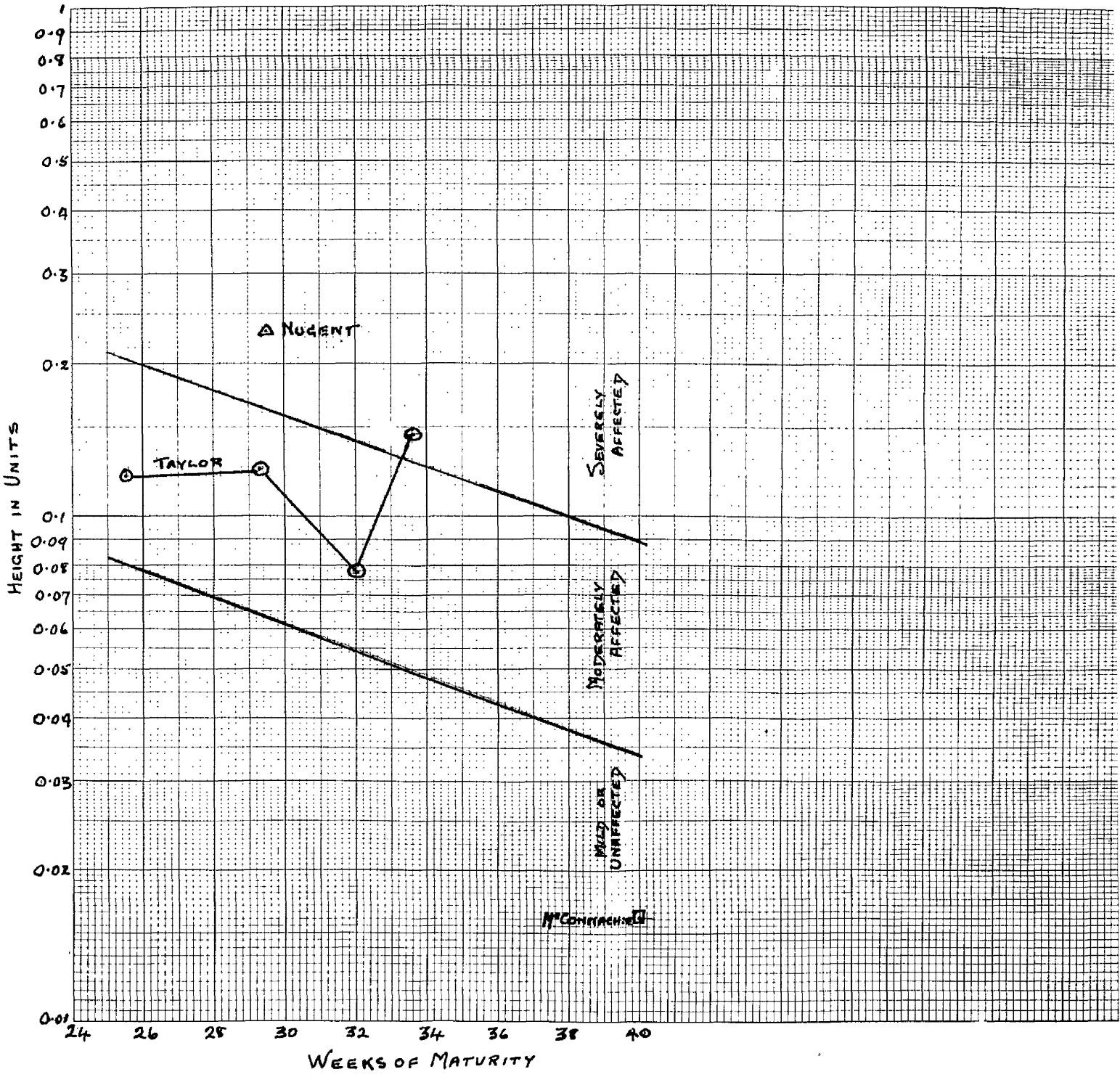

OPTICAL DENSITY
1
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
HAEMOGLOBIN PEAK
HAEMOGLOBIN PEAKS
ARTIFICIAL MIXTURE —
NORMAL LIQUOR
+
10 MGRMS. % HAEMOGLOBIN
360
400
450
500
550
600
650
700
HAEMOGLOBIN PEAK
BILIRUBIN PEAK
HAEMOGLOBIN PEAKS
ARTIFICIAL MIXTURE —
NORMAL LIQUOR
+
1 MGRM. % BILIRUBIN
+
10 MGRMS. % HAEMOGLOBIN
350
400
450
500
550
600
650
WAVELENGTH mμ

GRAPH 11



This is a Liley prediction graph showing the effect of meconium in the liquor amnii and its decrease.

GRAPH 111



This graph shows the three zones of a Liley type prediction graph.

GRAPH IV

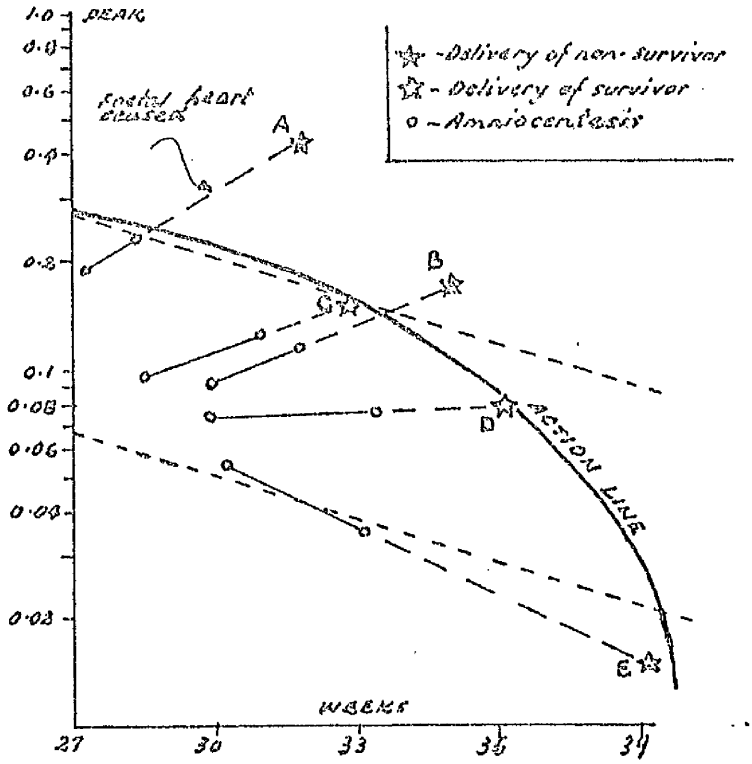
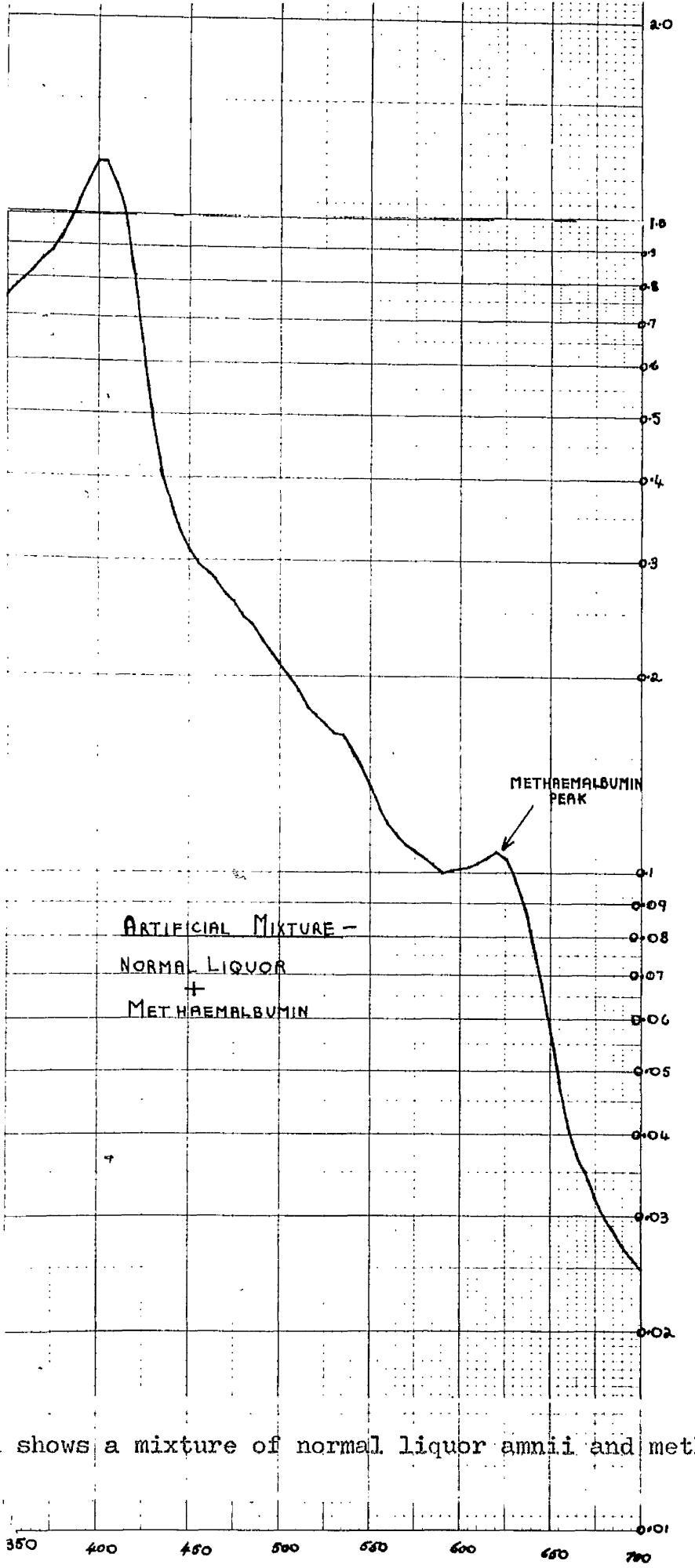


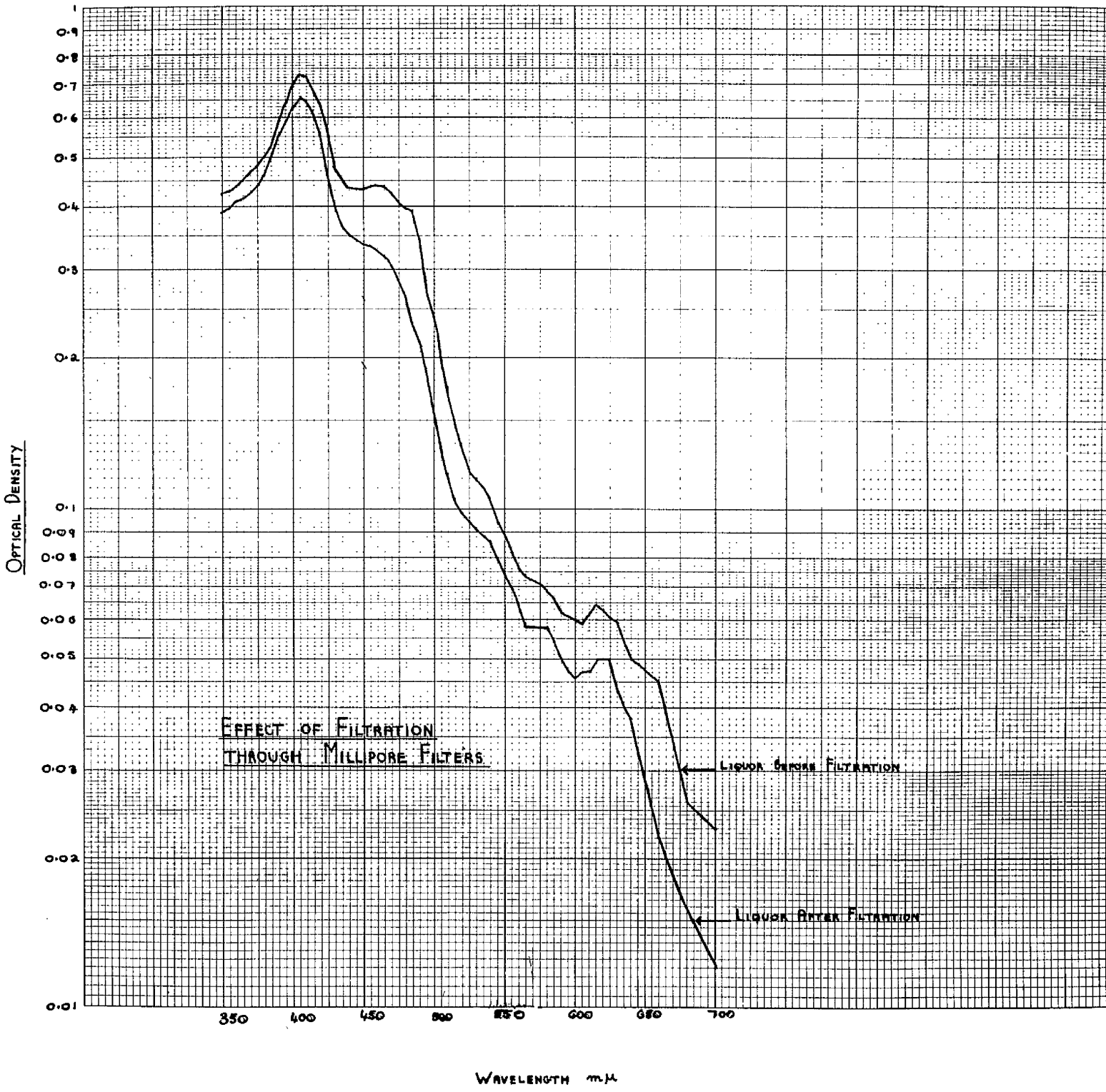
Diagram of action line (WHITFIELD, NEELY and TELFORD). J. Obst. & Gynaec. Brit. Comm. Vol. 75 P.121. 1968



GRAPH V

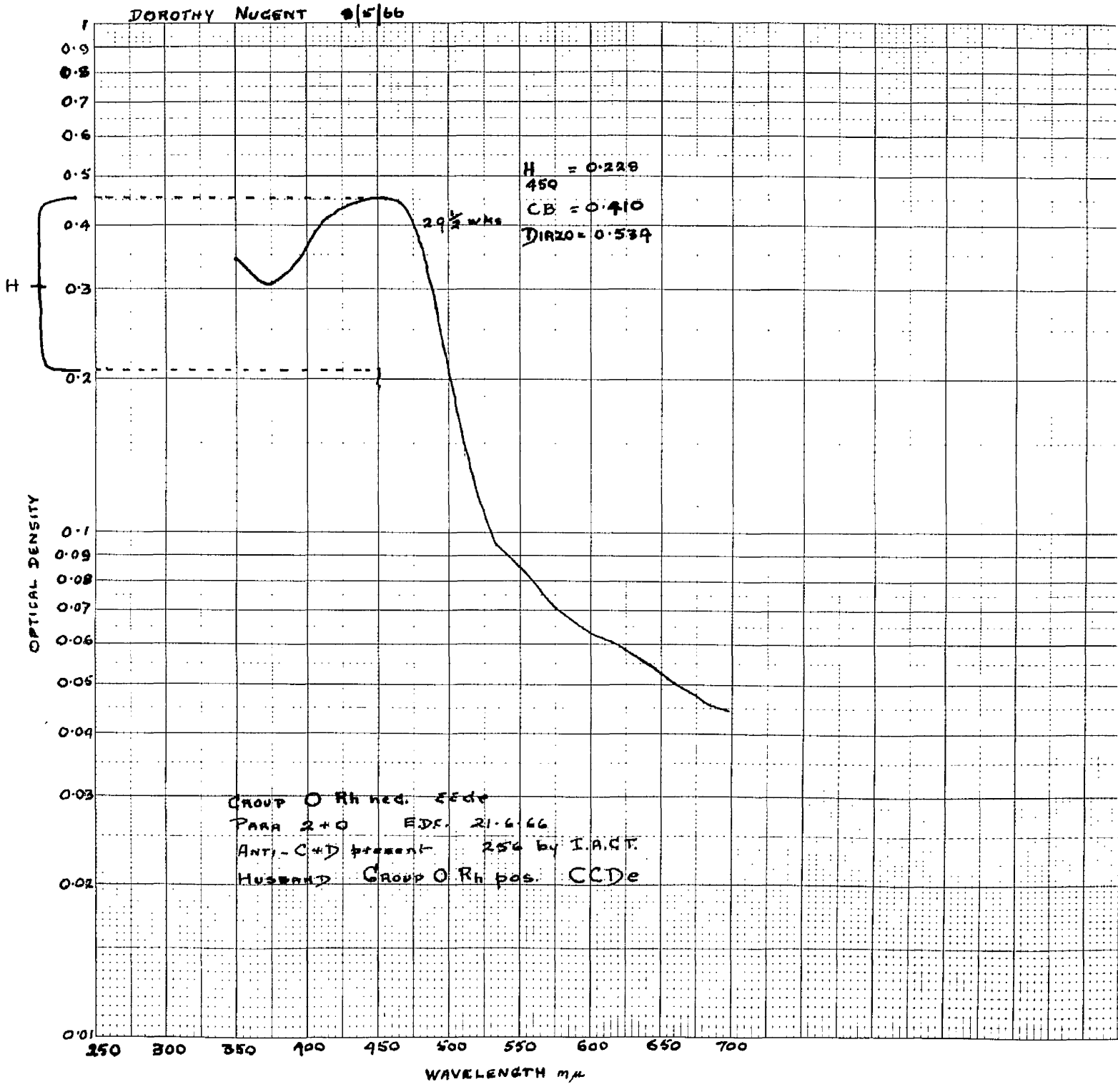
This graph shows a mixture of normal liquor amnii and methaemalbumen.

GRAPH V1



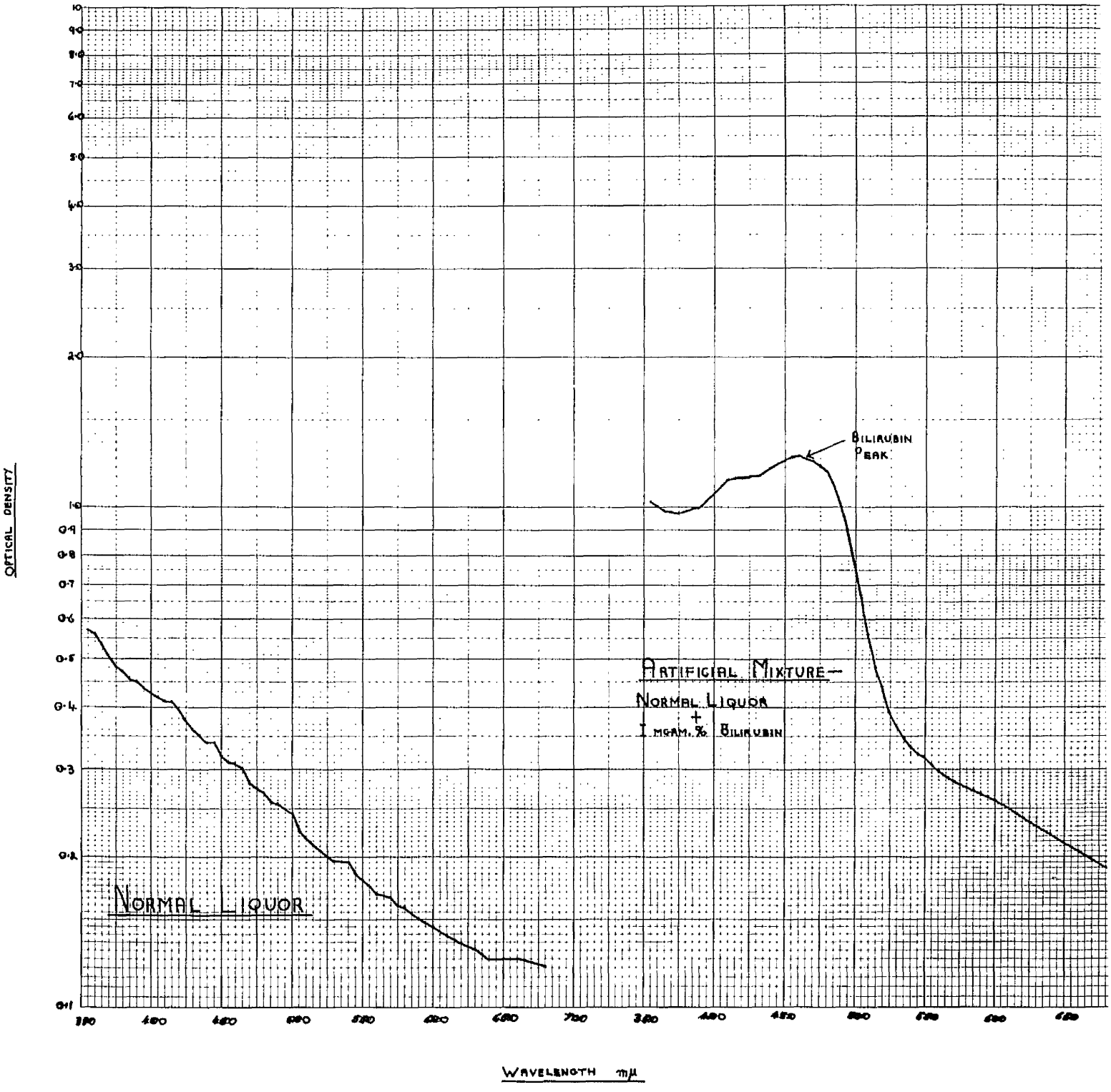
This graph shows the loss of bilirubin by filtration through Millipore filters.

GRAPH V11



This graph shows the calculation of the Optical Density Difference of bilirubin in an affected case.

GRAPH V111



This graph shows the spectrophotometric curves of a normal liquor and a normal liquor with added bilirubin.

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CHAPTER IV

INTRA-UTERINE TRANSFUSION

INTRA-UTERINE TRANSFUSION

The introduction of the technique of Intra-uterine Transfusion by Liley in 1963 shed the first ray of light on the dark obstetric future of the Rhesus immunized woman, with a Rhesus positive homozygous husband who had lost one or more babies from Haemolytic Disease of the Newborn. By making use of the fact, recognised in 1863 and first used clinically in 1875, (Friesen, R.F., Bowman, J.M. 1966) that intact red blood cells are absorbed from the peritoneal cavity via the diaphragmatic lymphatics and the thoracic duct to the venous circulation, it is possible to transfuse the foetus and treat the developing intra-uterine haemolytic anaemia thus preventing cardiac failure - the cause of intra-uterine death. Since 1963 this technique has been accepted where indicated as part of the management of the sensitized mother (Liley, 1964; Karnicki, 1966; Queenan, 1969; McCrostie, 1964; Fairweather, Tacchi, Coxon, Hughes, Murray and Walker, 1967).

Originally Liley thought that red cell absorption from the foetal peritoneal cavity took place at the rate of 12% per day and was complete eight to ten days after foetal transfusion. Creasman, Duggan and Lund (1966), by the use of chromium labelled cells, showed that there was almost 100% absorption in three days between intra-uterine transfusion and delivery. Intra-uterine transfusion is not, however, without risk to both mother and baby. It requires skilled operators and a close liaison between obstetrician, paediatrician, radiologist and laboratory worker. Experience and skill is only acquired with time and a series of cases. This is best developed by one team in

one unit and such cases requiring active treatment should be centralised in hospital where these teams are readily available.

MATERNAL RISKS

The dangers to the mother are varied. Placental separation and antepartum haemorrhage come immediately to mind in an operation of this type particularly when the placenta lies anteriorly and is in danger of being pierced by the needle. Infection is an ever present risk, especially when intra-uterine death follows rapidly after intra-uterine transfusion. Such a complication was reported by Horger and Hutchinson (1969) when hysterectomy became necessary following infection of the foetus and uterus by a gas-producing micrococcus. Infection may also take the form of amnionitis or peritonitis if amniotic fluid leaks back along the needle track. Friesen, Bowman, Barnes, Grewar, McInnis and Bowman (1967) report a case in which liquor amnii may have reached the maternal circulation from a false space when the membranes were partially detached from the uterine wall. As this fluid is rich in thromboplastic substances it could cause intravenous clotting with potential danger to the mother's life. Further anxiety may be caused to the operator by the knowledge that the mother may have had a previous Caesarean Section and thus the uterus is scarred and weakened. A late maternal risk, namely serum hepatitis, has been reported following foetal transfusion (Bishop, Weber and Israel, 1967; Wade, Ogden, Anderson and Davis, 1969).

FOETAL RISK

Many of the early fears of intra-uterine transfusion were centred

on the danger of trauma and haemorrhage in the foetus. Injury to the soft tissues - liver, spleen, abdominal and other organs - have been reported (Friesen et al, 1967 and Fairweather et al, 1967). Fairweather and others have reported injection of dye into the plural cavity, intestinal lumen, bladder, pericardium and even into the spinal canal with a resultant myelogram. If the spleen and liver are lacerated these injuries appear to heal well (Karnicki, 1968) and do not necessarily affect the outcome of the pregnancy. Karnicki (1966) also reports a case where a piece of catheter about 10 centimetres in length broke off in the foetal peritoneal cavity and was left there with no obvious permanent damage to the child which was born alive. Laceration of the umbilical cord has been reported causing foetal haemorrhage and gas gangrene to both mother and foetus (Queenan, 1969). Foetal mortality as a direct result of intra-uterine transfusion has been reported as high as 14.5% to 19.2% per transfusion by Friesen et al (1967).

During intra-uterine transfusion there may be foeto-maternal bleeding which may cause further stimulation of antibody production in the mother and thus cause a deleterious effect on the foetus. According to Liley (1964) this risk is academic. The blood used may well cause harmful effects to the foetus - serum hepatitis - as previously mentioned, cytomegalic inclusion disease (Karnicki, 1968) and injection of fresh leucocytes carrying the risk of runt disease (Naiman; Plunnet; Destine and Lischner, 1966). Depression of the foetal marrow may also result from intra-uterine transfusion. Schwarz (1967) in a letter to the Lancet reports two cases of apparent

haemorrhagic diathesis, from which the foetuses died, after transfusion with washed cells. He suggests that as treble washed cells are relatively free of all elements except erythrocytes, incoaguable haemorrhage in the foetus may be as much a danger as runt disease.

INDICATIONS FOR INTRA-UTERINE TRANSFUSION

While broad principles may be followed as a guide for intra-uterine transfusion each case must be judged on its individual merits.

The fundamental parameters leading to a decision to undertake intra-uterine transfusion are:-

(1) A history of previous pregnancies in which the foetus was severely affected by Haemolytic Disease particularly where the outcome was a stillbirth or neonatal death.

(2) The findings at amniocentesis where the amniotic fluid showed a high concentration of bilirubin either by spectrophotometric or biochemical methods - See previous chapter.

(3) The genotype of the husband particularly when he is thought to be homozygous Rh. D positive. The husband's genotype is of especial importance when the first pregnancy in which antibodies appear is just the patient's second pregnancy. The knowledge that all future

pregnancies will be affected due to the homozygosity of the father, always resulting in a Rhesus positive baby, lends weight to the decision for active intervention in such a case where the amniocentesis findings and antibody titre indicate a severely affected baby and previous history is lacking. These cases carry an 8% mortality before the 33rd week of pregnancy (Robertson, 1964). The zygosity of the father is conversely of value in a case where a woman has a history of previously badly affected pregnancies and yet has liquor findings suggestive of a mild or unaffected foetus. In this case the knowledge that the father is heterozygous Rh. D positive may strongly support the diagnosis of an unaffected foetus. The main pitfall in the use of the husband's genotype is where there is an extra-marital liaison - usually unknown to the obstetrician. It might also be mentioned that unless family studies are done the genotype is only a probable one and that errors in assumption are not unknown e.g. a CDe/cde genotype may be assumed where, in fact, it is CDe/cDe.

(4) ANTIBODY TITRE

With the advent of amniocentesis maternal antibody titre has played a slightly lesser role in foetal prognosis in Haemolytic Disease of the Newborn. A rising titre of maternal antibody suggests an affected foetus, the significant clinical level varying among several workers. This is well reviewed by Mollison (1967). Several factors may influence the rise of antibody titres and thereby invalidate the prognosis. The first is the anamnestic rise which may occur when a woman who has had a previously affected baby carries a Rhesus negative foetus. Such rises may be very sharp and reach levels of 1/8,000 by indirect antiglobulin technique but the answer

is usually found by liquor amnii examination. Amniocentesis and intra-uterine transfusion themselves may cause a fluctuation of antibody titre by provoking a foeto-maternal haemorrhage stimulating antibody production. This is one of the accepted risks of both procedures and is felt to be of little real importance (Walker et al, 1963). One definite use of antibody titre is to select cases for amniocentesis and therefore possible intra-uterine transfusion. In this series the antibody level used is 1/16 by indirect antiglobulin titre to indicate the necessity for amniocentesis but each laboratory has to find its own critical titre.

(5) CLINICAL FINDINGS

While laboratory aids to prognosis are essential the clinical findings in potential cases of intra-uterine transfusion are of paramount importance. It is accepted by all workers in this field - Liley (1963); Queenan (1969); Karnicki (1966); Friesen (1968) - that the selected time of gestation for intra-uterine transfusion is important. Where a stillbirth or neonatal death has resulted from a previous pregnancy it is usually necessary to intervene actively at an even earlier stage in a subsequent affected pregnancy. Should this occur before the 28th week of pregnancy intervention in the form of intra-uterine transfusion can be technically very difficult and the number of transfusions required may be as many as three or four or more, each carrying a 14.5% - 19.2% risk to the foetus (Friesen et al, 1967). In such cases where the signs of likely foetal death from Haemolytic Disease e.g. ascites, oedema, appear early i.e. before the 26th or 28th week, intra-uterine transfusion has a much lesser chance

of being successful and the clinician may wonder if the maternal risk is justified. This point will be further discussed later.

PRACTICAL TECHNIQUES OF INTRA-UTERINE TRANSFUSION

BLOOD USED

Blood collected in acid citrate dextrose and not more than 48 hours old is used. Preferrably the blood should be at least 12 hours old to cut down the risk of cytomegalic virus infecting the foetus. It is now possible, by testing the chosen blood for hepatitis associated antigen to reduce the risk of this particular infection in both mother and foetus.

The blood used is Group O Rhesus negative cde/cde largely free from anti A or anti B haemolysins and is compatible with the mother's serum. Five hundred and forty ml. of blood are centrifuged at 1,800 revs/min. for half an hour in a Mistral refrigerated centrifuge at $4^{\circ}C$. The plasma and buffy coat are then removed and saline added till the volume of cells and saline is 540 ml. This is mixed well and filtered through a blood giving set. The blood and saline mixture is again centrifuged at 1,800 revs/min. for half an hour following which the saline and remaining buffy coat are removed. Fresh saline is added to make up a total volume of 200 ml. approximately. This gives a concentration of red cells with a haemoglobin of 20-22 gms.%.

By taking into account the ABO, Rh., MNS, Kidd, Duffy, Lewis and

TECHNIQUE OF INTRA-UTERINE TRANSFUSION OF THE FOETUS

M. D. Black, W. H. Dempster and J. Y. MacDougall

Law Hospital, Carluke

SINCE Liley (1963) published the first report on intra-uterine transfusion of the foetus this procedure has been used in a number of centres and the technique has been modified in the light of experience by various authors.

As this treatment is now being adopted in many units a preliminary report of the technique used by the authors is submitted. This technique has been found to simplify the procedure, apparently increase its safety, and reduce the time involved.

On the day prior to transfusion amniocentesis is carried out and 20 ml. urografin 76 per cent is injected into the amniotic cavity.

On the day of operation the patient is sedated with sodium amytal, 200 mg. and Sparine, 50 mg. one hour before operation. The procedure takes place on the X-ray table. In a patient receiving a first transfusion a lateral X-ray is taken to localise the placenta using the 'water pillow' technique (Fig. 1). A skin marker is now affixed to the abdomen in the region of the umbilicus and the foetus is gently palpated.

The abdomen is then prepared with spirit and iodine and a sterile improvised grid is applied to the abdomen over the uterus as suggested by Lees (1966) and the abdomen is now draped with a sterile operating sheet.

An antero-posterior X-ray is taken and the plate is studied on the viewing box. A point vertically above the urografin shown in the foetal bowel below the level of the foetal liver and above the presumed position of the foetal bladder is chosen and its position relative to the grid is noted. The size of the squares on the grid is important as small squares diminish definition in the T.V. monitor. The optimum size is 1½-2 in. per square (Fig. 2). This point is now identified on the mother's abdomen and a local anaesthetic is injected into the skin and down to the peritoneum. An incision about ⅛in. is made over the point with a scalpel and the Tuohy 16 gauge 18 cm. needle is now passed vertically downwards till the foetal abdomen is encountered. Two assistants, one at the head of the table and one at the side of the table, guide the operator in keeping the needle vertical during insertion.

Fig. 1. Placental localisation film.

Fig. 2. Grid localisation of foetal bowel.

When the needle reaches the foetal abdominal wall slight resistance is encountered and then a distinct 'give' is felt as it passes into the peritoneal cavity.

A Portex intravenous catheter, length 26 in. is passed through the Tuohy needle and some inches beyond its point. An attempt is made to aspirate fluid and if no liquor amnii can be aspirated it is presumed that the catheter is now in the foetal peritoneal cavity. Urografin 76 per cent or Hypaque 45 per cent 3 to 4 ml. are injected through the catheter and the area is now briefly viewed on the image intensifier. If the foetal abdomen is not clearly outlined a further 1 to 2 ml. opaque medium are injected under vision with the image intensifier. If the radio opaque substance remains localised and the curve of the anterior abdomen and diaphragm visualised then the catheter is considered to be in the foetal peritoneal cavity. In the early cases with this technique a further antero-posterior X-ray film was taken after the injection of the 3 to 4 ml. urografin or Hypaque (Fig. 3).

According to the maturity of the foetus, 80 to 120 ml. of warmed, washed packed cells of group O Rhesus negative blood, haemoglobin 20 g. per cent, are injected using a two-way 10 c.c. syringe. The blood is held in a giving set and stand, and as the catheter is very fine, it has been found to be easier to make the injection through the relatively narrow bore of a 10 c.c. syringe.

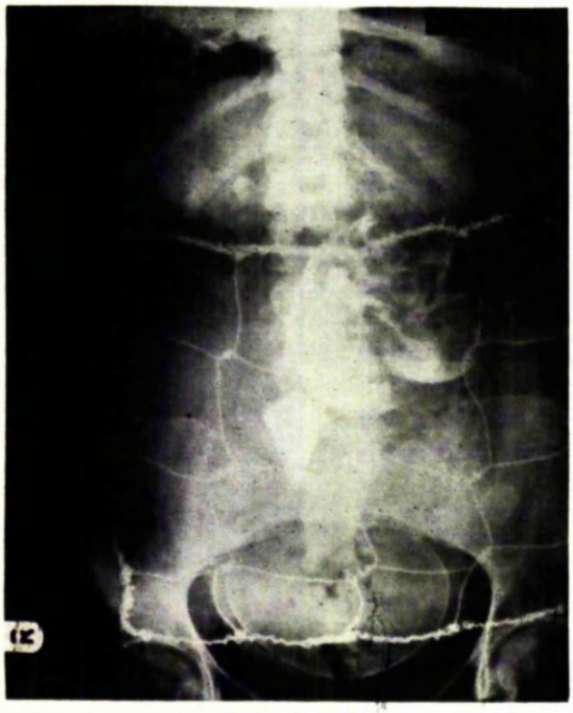

Fig. 3. Foetal abdomen outlined with dye.

At the end of the procedure the needle and catheter are removed and the abdominal wound sealed with collodion and a gauze swab.

With the technique described it has been found possible to carry out the procedure in about 30 to 40 minutes. The use of the grid and the vertical insertion of the needle usually enables the target area to be reached at the first attempt. One hazard is that an anteriorly situated placenta may have to be traversed but this is a risk of all procedures when the placenta is so situated.

Radiation dosage is carefully controlled. The average screening time with the low dosage image intensifier and T.V. monitor is 1½ minutes and average number of films per examination is 2.

Case s

Fifteen patients have been treated by 26 intra-uterine transfusions, the maturity of the foetus varying between 26 and 32 weeks. The first 5 cases were treated without the image intensifier and grid, and only 1 baby survived. The next 10 patients were treated using the image intensifier and 8 of the babies have survived. One of the 2 babies lost, died during a second exchange transfusion reflecting a hazard of exchange transfusion rather than the severity of the haemolytic disease.

One of the 15 patients has been counted twice as she has been treated in 2 pregnancies and the baby has survived on each occasion.

Two additional patients are at present under treatment.

During the last 12 intra-uterine transfusions the grid has been used and this has greatly simplified the procedure.

ACKNOWLEDGEMENT. We are grateful to Dr R. A. Tennent for his co-operation.

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Kell groups it has been possible to select blood distinguishable from the parents and sometimes from another donor used for a subsequent transfusion. By using these antigens for identification in association with the mixed agglutination technique (Jones and Silver, 1958) it has been possible to differentiate donor and foetal blood in the baby after delivery. Using the same techniques of identification blood which may have escaped into the liquor amnii or which may have been retained in the foetal peritoneal cavity may be grouped.

The use of maternal blood for transfusing the foetus has been described recently by Eyster, Queenan and Haber (1969). This method eliminates the risk of maternal homologus serum hepatitis and isoimmunisation to other blood group factors. The authors indicate that neither transfer of maternal Rhesus antibody to the foetus nor potential foeto-maternal ABO incompatibility is hazardous to the foetus. Precautions in the use of this method are that the maternal haematocrit must be 35% or greater and that no more than 150 ml. of blood are withdrawn for each transfusion.

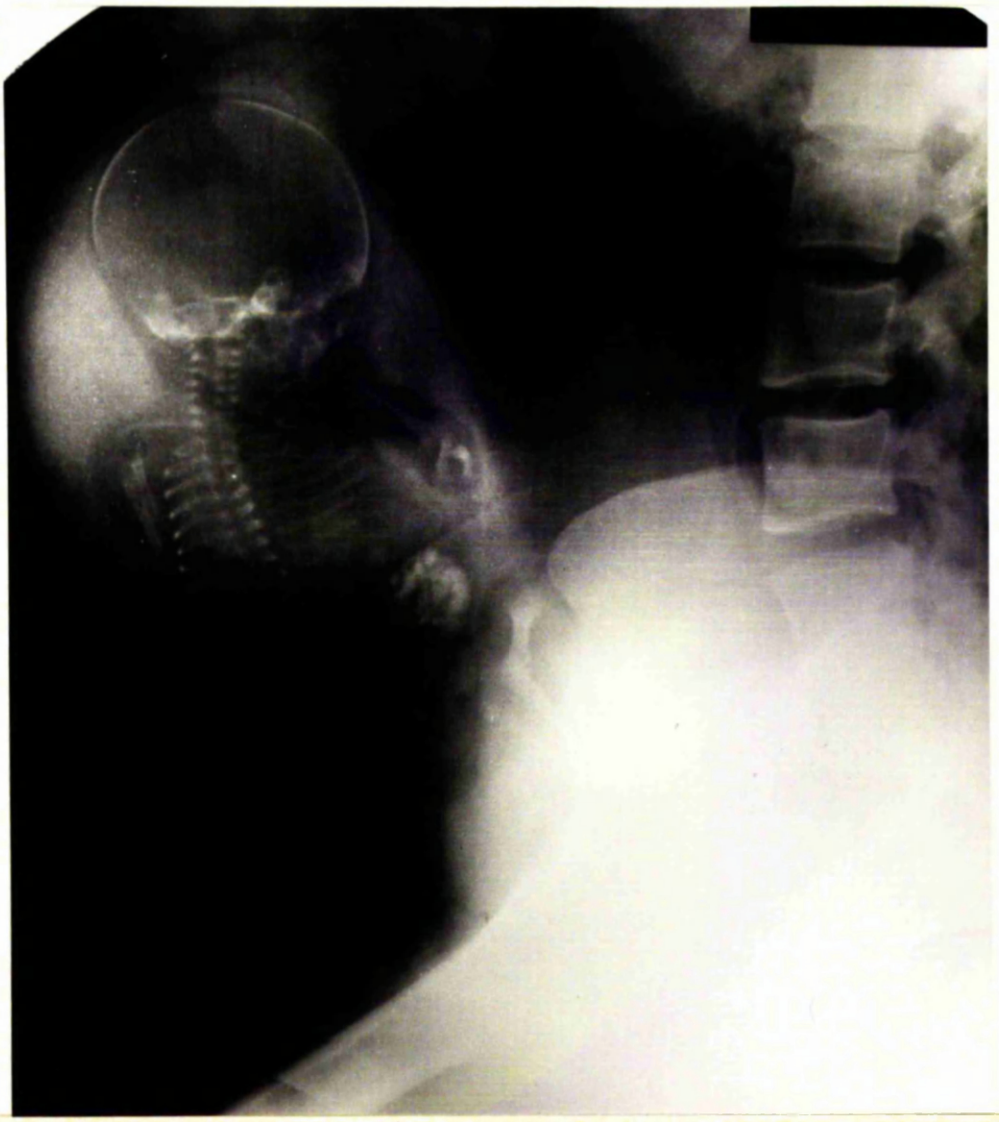
TECHNIQUES OF INTRA-UTERINE TRANSFUSION

TRANS-ABDOMINAL TECHNIQUE

This is based on the method used by Liley (1963) and is the one used in this series. (Black, Dempster and MacDougall, 1967).

The patient is admitted to hospital on the day prior to the

PLATE 1



Lateral soft tissue x-ray film to show position of placenta and depth of radio opaque dye from anterior abdominal wall.

transfusion and amniocentesis carried out, 20 millilitres of 45% Hypaque being injected into the amniotic cavity. When it is the first intra-uterine transfusion to be given, some twelve hours later a soft tissue lateral x-ray of the woman is taken to localise the placenta. On the day of the transfusion the patient is sedated with Sodium Amytal, 200 mgm. and Sparine, 50 mgm. one hour before operation and the patient is transferred to the x-ray department where the transfusion takes place.

PLATE 1

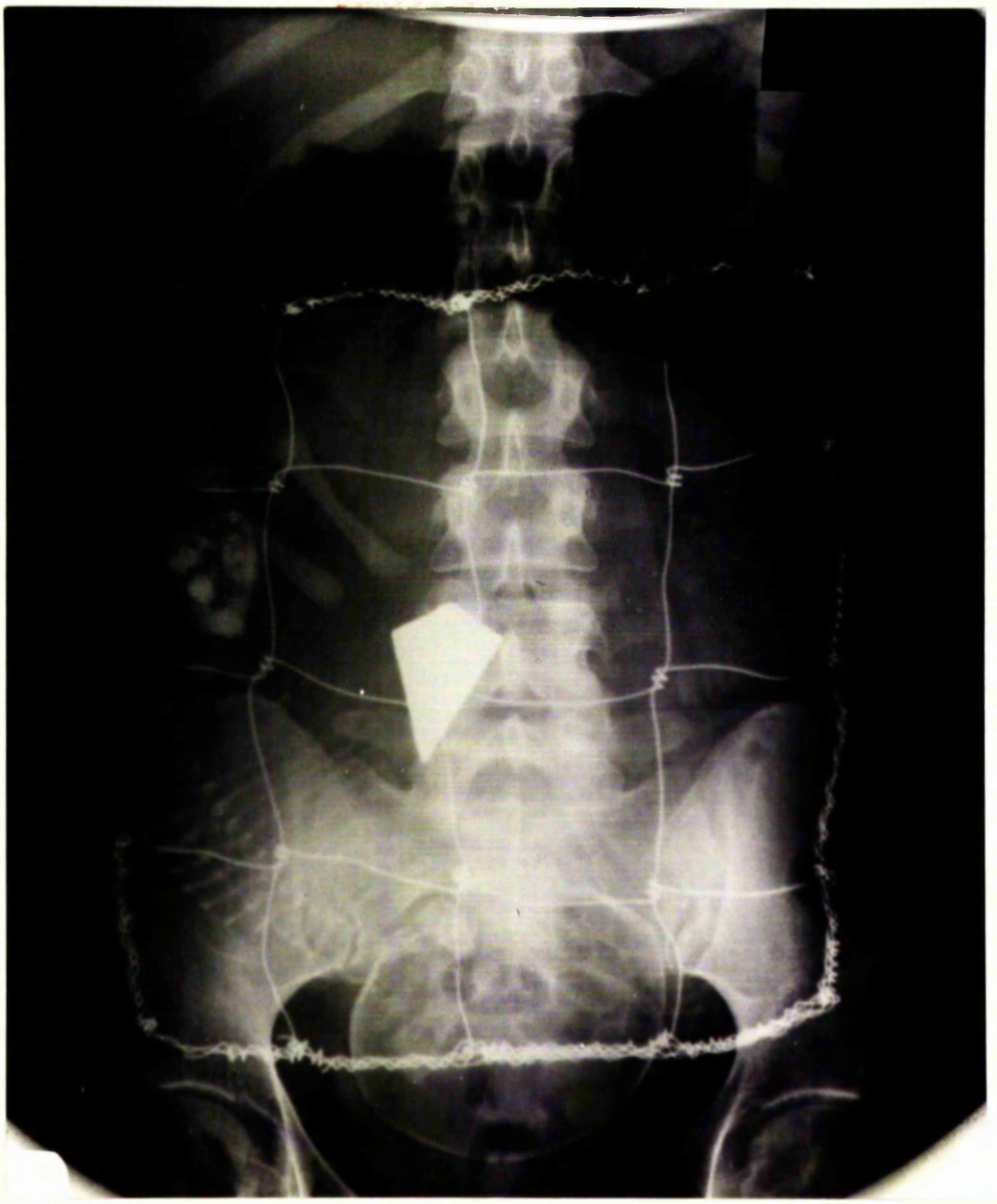
This shows the position of the foetus with the placenta lying posteriorly and the dye present in the foetal bowel.

This film helps to give guidance to the depth of the target area from the skin of the abdominal wall.

The sophisticated technique of localisation by isotopes and thermography are not yet available in Lanarkshire but, due to excellent radiology, the localisation of placentae is remarkably accurate.

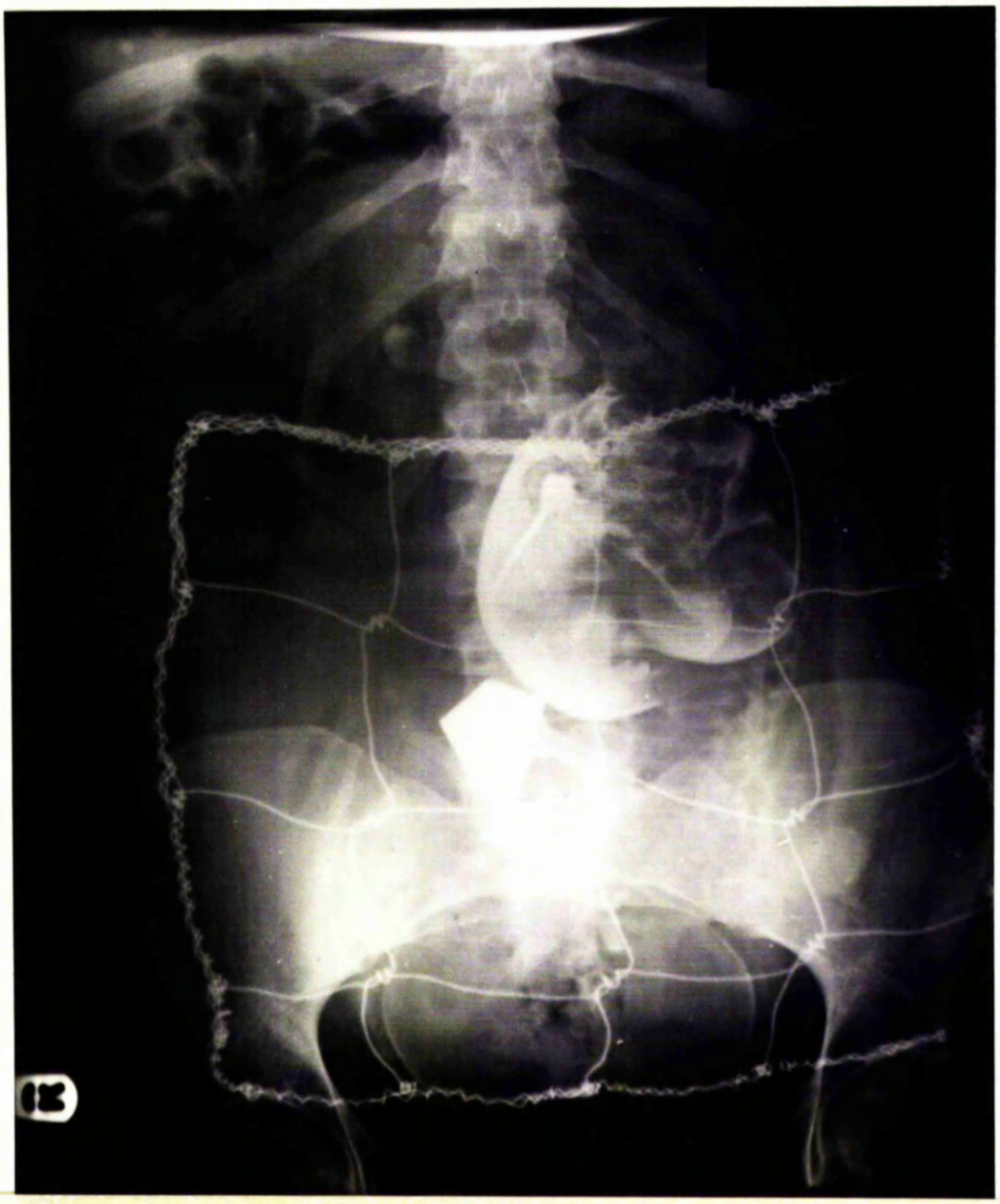
The patient lies supine for 15-20 minutes and then the foetus is gently palpated. The abdominal skin is then prepared with spirit and Iodine and a sterile grid of plastic covered wire is applied over the uterus as suggested by Lees (1966). The abdomen is now draped with a sterile operating sheet.

PLATE 11



This shows grid in position and relation of target area to squares in grid.

PLATE 111



This plate shows the needle and catheter in position with the foetal diaphragm and abdominal wall outlined in Hypaque. It also shows a marker over the maternal umbilicus which is no longer used.

An antero-posterior x-ray is taken and the plate is studied on the viewing box. A point for entry of the Tuohy needle judged to be vertically above the Hypaque shown in the foetal bowel, allowing for the x-ray distortion, below the level of the foetal liver and above the presumed position of the foetal bladder, is chosen and its position relative to the grid is noted. The size of the squares on the grid are important as small squares diminish definition on the Television Monitor. The optimum size is $1\frac{1}{2}$-2 inches square.

PLATE 11

This shows the grid in position and the relation of the foetal abdomen to the squares on the grid.

The selected point is now identified on the mother's abdomen and a local anaesthetic (1% Lignocaine) is injected into the skin and down to the peritoneum. A skin incision of about 1/8 inch is made over the point with a scalpel and the Tuohy 16 gauge 18 centimetre needle is now passed vertically downwards through the abdominal incision until the foetal abdomen is encountered. Two assistants, one at the head of the table and one at the side of the table, guide the operator in keeping the needle vertical during insertion. When the needle reaches the foetal abdomen slight resistance is encountered and then a distinct "give" is felt as it passes into the foetal peritoneal cavity. There may then be a definite movement of the needle indicating foetal protest.

A Portex intravenous catheter 26 inches in length is passed through the Tuohy needle and some inches beyond the needle end. An attempt is made to aspirate liquor and if no liquor amnii is obtained it is presumed that the catheter is now in the foetal peritoneal cavity. On occasions when foetal ascites is present and the needle is in the peritoneal cavity as much of the ascitic fluid is withdrawn as possible. The ascitic fluid is clear and very yellow and on the first occasion when it is withdrawn uncontaminated by blood, is easily distinguished by the naked eye from amniotic fluid. Two to three millilitres of 45% Hypaque are now injected through the catheter and the area is briefly viewed on the image intensifier. If the foetal peritoneal cavity is not clearly outlined a further 1-2 millilitres of opaque medium are injected under vision with the image intensifier. If the radio opaque material remains localised and the curve of the anterior abdominal wall is visualised the catheter is considered to be in the foetal peritoneal cavity. In the early cases with this technique a further antero-posterior x-ray film was taken after the injection of 2-3 millimetres of Hypaque but this is not considered necessary now.

PLATE 111

This plate from an early case in the series shows the needle and catheter in position with the foetal diaphragm and abdominal wall outlined by the Hypaque.

An attempt is made to withdraw some Hypaque from the abdominal cavity and then preparation is made to inject the prepared blood.

According to the maturity and estimated size of the foetus 40-120 millilitres of warm washed packed cells of Group O Rhesus negative blood concentrated to a haemoglobin of 20 gms.% are injected through the catheter using a three-way Tap and a 10 millilitre syringe. The blood is held in a giving set, and as the catheter is very fine, it has been found to be easier to introduce the blood using the relatively narrow bore of a 10 millilitre syringe taking approximately half a minute per millilitre. At the end of the procedure the needle and catheter are removed and the abdominal wound sealed with collodion and a gauze swab.

The use of the grid and the vertical insertion of the needle usually enables the target area to be reached at the first attempt. One hazard is that an anteriorly situated placenta may have to be traversed but this is a risk of all techniques when a placenta is so situated. Provided that only one clean puncture is made through the placenta, there appears to be little risk to mother or baby and less risk than multiple punctures at an angle in an attempt to avoid the placenta (Liley, 1969 - Personal communication).

Radiation dosage is carefully controlled. The average screening time with the low dosage image intensifier and Television Monitor is 1½ minutes and the average number of films per transfusion is two.

OTHER METHODS OF INTRA-UTERINE TRANSFUSION

TRANS-ABDOMINAL AMNIOSCOPY AND TRANSFUSION (WADE, OGDEN, ANDERSON AND DAVIS, 1969)

In two patients Wade and his colleagues used a modified paediatric fibre optic cystoscope 4 millimetres in diameter to place a needle into the peritoneal cavity of a foetus. The procedure was performed under local anaesthesia. The cystoscope was inserted through a thin-walled sheath into the amniotic cavity and a collapsible latex balloon, previously fitted over the end of the cystoscope, was inflated with air thereby pushing aside the turbid amniotic fluid. The umbilical cord was located and traced to its foetal origin. The Tuohy needle was then directed into the lower abdomen of the foetus under direct vision.

OPEN OPERATIVE TECHNIQUE

This has been described by Freda and Adamson (1966); Asensio, Figueroa-Longo, Pelegrina (1966) and Seelen, van Kessel and Been (1970). In this method the placenta was localised using radioactive isotopes and the abdomen was opened. A small incision was made in the uterus opposite a foetal foot and the membranes freed but not incised. The foetal foot surrounded by bulging membranes was brought out and the membranes incised. The leg was delivered as far as the groin and a purse-string suture put round the groin catching membranes and uterus so that as little amniotic fluid as possible escaped. The femoral artery was cannulated and 220 millilitres of fresh Group O Rhesus negative blood given by exchange transfusion. The limb was replaced in the uterus and membranes, uterus and abdomen closed. In the case of Freda and Adamson the procedure was well tolerated by both mother and foetus but two days later precipitate labour followed and a 27 week baby

was delivered which died in the neonatal period from atelectasis. Asensio and his colleagues reported the procedure with slight modifications in particular using the femoral vein. They successfully delivered a Porto Rican woman who had no living children of a live female child which survived.

A further series of open operative intra-uterine transfusions was reported by Seelen et al at the International Rhesus Symposium in Milan in October, 1969 with depressing results.

A further variation of the open operative technique is described by Wade and his colleagues (1969). In their series of three cases, hysterotomy was performed on patients with foetuses of less than 25 weeks' gestation, the foetus was delivered from the uterus and a silastic catheter inserted into the abdominal wall of the foetus and secured with sutures. The foetus was returned to the uterus, the catheter brought through a separate wound in the side of the uterus and the first transfusion performed. The membranes were then closed and the amniotic fluid replaced with sterile saline. In none of the three cases was a living child obtained.

DISCUSSION OF PRESENT SERIES OF INTRA-UTERINE TRANSFUSIONS

A total of 24 patients were treated involving 26 pregnancies with 48 transfusions. Ten babies have survived and are well following the procedure, with one exception which will be discussed later.

PREVIOUS HISTORY

This is summarised on Table 1.

One patient had three stillbirths, one had three stillbirths and one neonatal death, one had two stillbirths, four patients had one stillbirth and two had one neonatal death. Ten of the remaining patients had a history of babies requiring exchange transfusion. Those patients who had a previous stillbirth had an 80-90% chance of losing a child in this pregnancy and of all patients who have had a baby requiring exchange transfusion there is a 29% chance of intra-uterine death (Mollison, 1967). It is difficult to estimate the risk of intra-uterine death in the two patients who received intra-uterine transfusions subsequent to a previous pregnancy with intra-uterine transfusion but the risk must be very high.

LIQUOR FINDINGS

These are illustrated in Figure 1.

All the liquor results were, at the initial examination, in Zone 3, or entered Zone 3, of a Liley type prediction graph after serial examinations. Three cases, 5, 16 and 22, started in Zone 2. On liquor findings alone one might in retrospect criticise the extreme step of intra-uterine transfusion in some cases. In case 12 only one specimen of liquor was examined but was in Zone 3 and the patient had a history of a baby requiring multiple exchange transfusions. In case 5 the liquor optical density started in Zone 2 and only reached

TABLE 1

Case No.	Parity	Genotype of husband	Previous S.B. or N.N.D.	Previous exchange	Outcome
1	10 + 1	cDE/cde	3 S.B. 1 N.N.D.	-	S.B.
2	4 + 2	cDE/cDe	2 S.B.	-	S.B.
3	1 + 1	CDe/CDe	-	-	N.N.D.
4	7	cDE/cDE	-	1	S.B.
5	1 + 1	CDe/CDe	-	-	A & W
6	2	CDe/CDe	-	-	A & W
7	4	CDe/cDE	3 S.B.	-	A & W
8	3	CDe/CDe	-	2	A & W
9	1	CDe/cDE	-	1	A & W
10	2	CDe/cDE	-	1	S.B.
11	1	CDe/cde	-	-	N.N.D.
12	2	cDE/cde	-	1	A & W
13	6	CDe/cDE	1 N.N.D.	1	A & W
14	3	CDe/CDe	-	1 I.U.T.	A & W
15	3	CDe/CDe	-	1	S.B.
16	2 + 2	Not done	1 S.B.	-	A & W
17	2	cDE/cde	-	1	A & W
18	5	cDE/cde	1 S.B.	1	N.N.D.
19	2	cDE/cde	1 S.B.	1	S.B.
20	3	cDE/cde	-	1	N.N.D.
21	2	CDe/cde	-	1	N.N.D.
22	8	cDE/cDE	1 S.B.	1 exchange 1 I.U.T.	N.N.D.
23	3	CDe/cDE	1 N.N.D.	1	S.B.
24	3	CDe/cde	-	-	S.B.
25	4 + 2	cDE/cde	-	-	S.B.
26	3	CDe/CDe	-	1	N.N.D.

KEY - S.B. - Stillbirth N.N.D. - Neonatal death
A & W - Alive and well I.U.T. - Intra-uterine transfusion

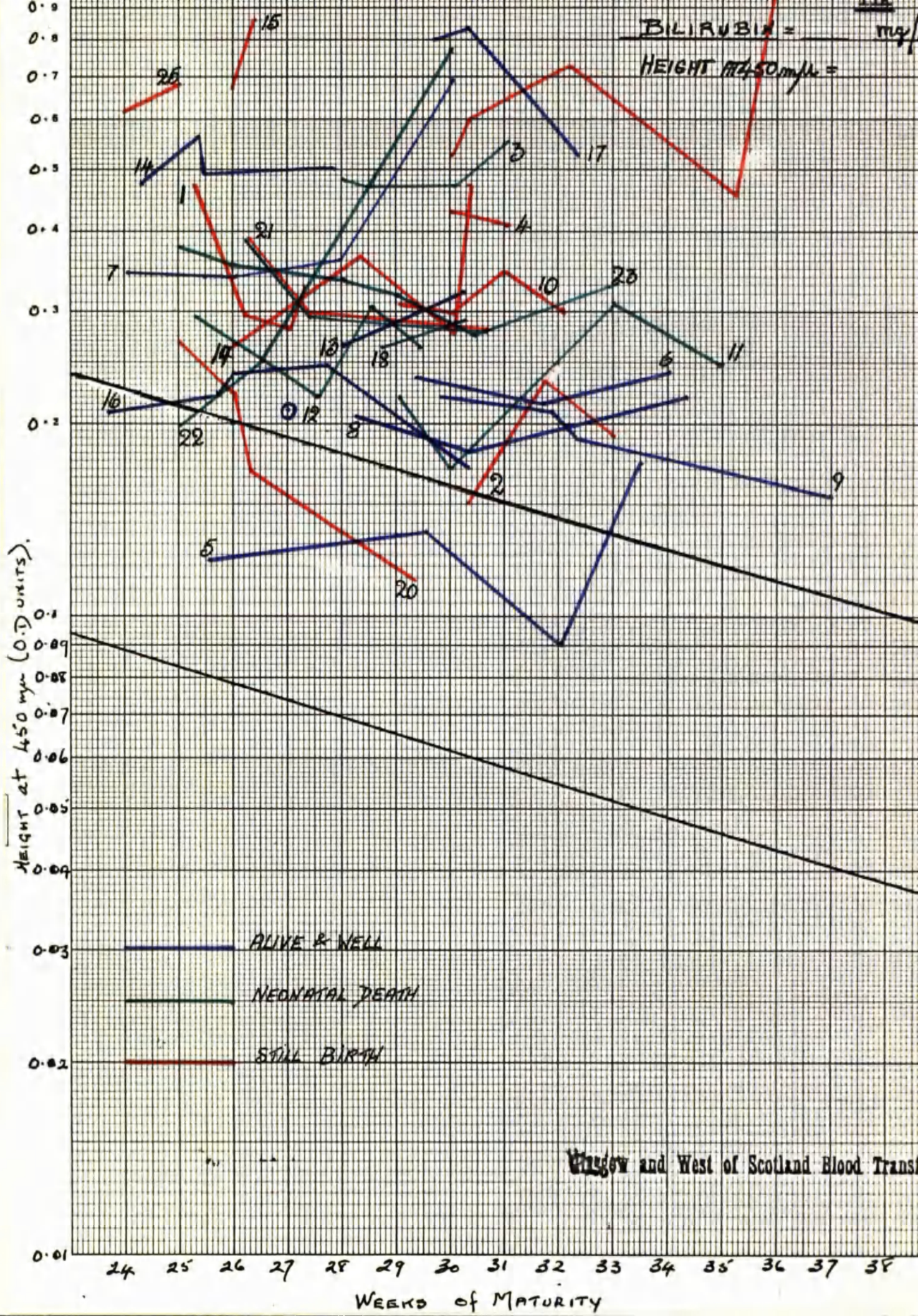

BILIRUBIN = ____ mg%
HEIGHT AT 450 mμ =
HEIGHT at 450 mμ (O.D. UNITS).
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0·09
0·08
0·07
0·06
0·05
0·04
0·03
0·02
0·01
ALIVE & WELL
NEONATAL DEATH
STILL BIRTH
24 25 26 27 28 29 30 31 32 33 34 35 36 37 38
WEEKS of MATURITY
Glasgow and West of Scotland Blood Transf

TABLE III (PAGE 113)
AT THE BACK OF THESIS.

Zone 3 on the last examination but again there was a previous history of a child requiring exchange transfusion. In case 20 the liquor optical density dropped to Zone 2 after intra-uterine transfusion at 26 weeks but intra-uterine death occurred in 3 days. All the other liquor findings were strong indications for intra-uterine transfusion.

RESULTS

These are summarised in Tables 1 and 11.

Of the 16 babies which did not survive, 7 were neonatal deaths. One died of a cardiac arrest during a second exchange transfusion; the others were hydropic at delivery and failed to respond to immediate resuscitation and transfusion. The other 9 infants lost, died in utero. Autopsy was performed on all but one of these but the findings were difficult to interpret because of the degree of autolysis. All those examined showed histological evidence of severe erythroblastosis foetalis in liver and spleen. Examination of cord or heart blood was unfortunately not possible due to haemolysis. In none of these cases was there obvious trauma from the intra-uterine transfusion. The time interval between the last intra-uterine transfusion and intra-uterine death was a minimum of three days and a maximum of nine days - a significantly short interval. One patient had a placenta praevia and had antepartum haemorrhages which no doubt did contribute to the unfortunate outcome. One patient had a massive accidental haemorrhage three days after intra-uterine transfusion and delivered

a stillborn foetus. It is interesting to note that she had an equally massive accidental haemorrhage with a stillborn foetus in a previous pregnancy without intra-uterine transfusion. Another patient also had an accidental haemorrhage.

This total of 9 intra-uterine deaths from 48 transfusions gives a maximum risk of 18.8% with each transfusion if all deaths are caused by the transfusion. Such a risk is high especially if the risk is cumulative when 2 or 3 transfusions are necessary but without intra-uterine transfusion all those babies would probably have died. However, there was no definite evidence that these deaths were a result of the intra-uterine transfusion.

The 10 surviving babies required either exchange transfusion or simple top-up transfusion. The mean cord blood findings were - Haemoglobin 10.1 gm.% and serum bilirubin 6 mgm.%. The amount of adult blood in the cord blood varied from 30% to 100% using the mixed agglutination technique and the acid elution technique. In cases 14 and 16 the percentage of adult blood in the cord was 100%. In both these cases no exchange transfusion was necessary - only simple top-up transfusions - as the following detailed descriptions show.

CASE 14

This baby was reported as being Group O Rhesus negative with a cord haemoglobin of 12.4 gm.% and serum bilirubin of 3.1 mgm.%.

The direct Coomb's test was negative. All the cells appeared to be of the adult type. Anti D was detectable by both saline and albumen techniques. Date of birth was 15.5.67. Anti D remained detectable by the enzyme technique until 14.8.67. the titre at birth being 1/64 and the Coomb's test remained negative until 13.7.67 when about 25% of the cells were Coomb's positive. During this period the haemoglobin fell slowly to 8 gm.% and the infant was given two top-up transfusions. The serum bilirubin never rose above 13 mgm.%.

CASE 16

A similar picture was found in this baby. At delivery on 29.7.67 the cord blood was grouped as O Rhesus negative with a haemoglobin of 13.6 gm.% and a serum bilirubin of 3.4 mgm.% and all the cells appeared to be of the adult type with a negative direct Coomb's test. Anti D was present in the baby's blood to a titre of 1/8 by enzyme technique. The bilirubin never rose above 8.2 mgm.% although the haemoglobin dropped slowly over eight weeks necessitating two top-up transfusions. On 26.9.67 the direct Coomb's test was again negative but a positive result was obtained by using the Jones and Silver technique (1958). Anti D remained present in the infant's blood in minute quantities until the same time.

Both cases illustrated the potential of great success with intra-uterine transfusion. Each had a very high percentage of donor cells present at birth and the need for exchange transfusion was avoided. While it is realised that many factors play a part in

the production of the blood picture in these infants it can be seen that the transfused adult celle seem to have a life of 2-3 months. What part the donor blood, given by intra-uterine transfusion, plays in the suppression of the child's own marrow and what part the anti D antibody plays in haemolysing the few red cells produced by the baby in the first few weeks of life remain matters for conjecture.

RELATION OF INTRA-UTERINE TRANSFUSION TO MATURITY

This is summarised in Table 111.

Neonatal mortality in infants with Haemolytic Disease induced at 32 weeks' gestation can be 40% or higher compared with 20% mortality for those induced at 35 weeks (Robertson, 1964 and Walker et al, 1957). Intra-uterine transfusion based on liquor examinations in the mid-trimester of pregnancy and previous history, would seem to remove the need for such early induction. It is, however, true that early intra-uterine transfusion e.g. at 24 weeks or earlier, is technically more difficult than those performed at 30 weeks' gestation or later. In addition such an early start means that 2, 3 or 4 transfusions are necessary to prolong gestation until the baby is mature enough to have a good chance of survival. This, of course, means exposing the baby to the cumulative risks of intra-uterine transfusion. Thus it is not surprising that the overall salvage rate at 26 weeks or earlier is only 20% (Queenan, 1969).

In the present series 12 of the 26 babies were transfused

TABLE 111

Transfusion related Maturity

	Time of initial transfusion in weeks						
	26-27	27-28	28-29	29-30	30-31	31-32	32-33
All cases	3	1	3	5	5	5	4
Survivors	2	-	-	2	2	2	2

This table shows the maturity of the foetus at the time of the initial transfusion.

TABLE 1V

Treatment of Survivors

Cases	No. of Exchange Transfusions			Top-up alone
	1	2	3	
10	1	4	3	2

This table shows the number of exchange transfusions and top-up transfusions in the surviving infants.

before 30 weeks with a survival rate of 33%. The latest initial transfusion was at 32½ weeks and of those later transfusions 42.8% survived. The later the initial transfusion can be carried out the better the chance of survival due to the lesser risk of the procedure and the lesser degree of affection of the baby. In very late transfusions i.e. at 35 weeks or more, the doubt is raised whether in fact intra-uterine transfusion is really necessary or whether it would be safer than immediate delivery. With improved technique and further experience early transfusions should produce better results. In the present series there is an overall survival rate of 38.5% which is comparable with most international series (Queenan, 1968; Karnicki, 1969; Friesen et al, 1967; Fairweather et al, 1967; Wade et al, 1969 and Liley, 1970).

PRESENCE OF HYDROPS FOETALIS IN RELATION TO THE USE OF INTRA-UTERINE TRANSFUSION

There was much discussion at the Rhesus Symposium in Milan (1969) about the cause of hydrops foetalis and the effect of intra-uterine transfusion on such a baby. It was agreed that when the full hydropic syndrome had not developed and only ascites was present then intra-uterine transfusion could be of benefit. In the present series this was found to be true. In cases 16 and 14 ascitic fluid was withdrawn at the first transfusion and both babies survived. In case 3 ascitic fluid was also withdrawn but oedema of the foetus became apparent on both x-ray films and the baby was hydropic at delivery and lived only 48 hours. In none of the other cases was ascites or hydrops demonstrated at the time of intra-uterine transfusion. This experience supports other workers who feel that

once the full hydrops syndrome has developed whatever the cause - profound anaemia or liver failure, the outlook even with intra-uterine transfusion is hopeless. Indeed, in view of the maternal risks it is doubtful whether it is justifiable even to attempt intra-uterine transfusion on a clinical hydrops when the results are so discouraging. When, however, only ascites is present intra-uterine transfusion can be a worthwhile measure.

CAUSES OF FOETAL AND NEONATAL LOSS

This is summarised in Table V.

Total number of stillbirths was 9. One of these was unconfirmed as erythroblastosis due to autolysis of the foetus making histological examination difficult. Of the others one was associated with a placenta praevia and antepartum haemorrhage. Two others were associated with mixed accidental haemorrhages. All of these babies showed histological evidence of erythroblastosis foetalis.

Of the neonatal deaths, one had a cardiac arrest during a second exchange transfusion, a recognisable risk of exchange transfusion and in no way attributable to intra-uterine transfusion, and one was delivered at 29 weeks by elective Caesarean Section because of maternal eclampsia. This was a very premature baby showing signs of erythroblastosis which survived for only a few hours. The others were all hydropic and failed to respond to resuscitative measures and exchange transfusion.

TABLE V

PERINATAL MORTALITY

Cause of Neonatal Deaths

Hydrops foetalis alone	4
Hydrops foetalis + Maternal eclampsia	1
Hydrops foetalis + Respiratory Distress Syndrome	1
Cardiac Arrest during exchange transfusion	1
TOTAL	7

Cause of Stillbirths

Hydrops foetalis alone	5
Erythroblastosis + Placenta Praevia	1
Erythroblastosis + Mixed Accidental Haemorrhage	2
Unexplained intra-uterine death	1
TOTAL	9

FATE OF SURVIVORS

With one exception all the survivors are normal children in every way, having passed the milestone of development at times comparable to children who have not had their unusual foetal and neonatal background.

The child who has not progressed normally was delivered by Caesarean Section at 35 weeks having had one intra-uterine transfusion. She was in good condition at birth with a cord bilirubin of 6.2 mgm.% and a haemoglobin of 10.2 gm.%. Exchange transfusion was carried out one hour after delivery and repeated 24 hours later because of hyperbilirubinaemia. During the second exchange transfusion she had a cardiac arrest but responded to prompt and intensive resuscitation. A further exchange was carried out on the third day and progress seemed normal in the neonatal period. When re-assessed at six months she was noted to be developing slowly, and subsequent examination confirmed the diagnosis of spasticity. She is now attending a day centre for handicapped children and is making slow but sure progress although obviously mentally handicapped as well. Whether the cause was kernicterus or the result of the severe anoxic episode is uncertain.

METHOD OF DELIVERY

Of the 26 cases in the present series, 18 were delivered by Caesarean Section and 9 of these babies survived; there were 6 neonatal deaths and 3 stillbirths. Eight babies were spontaneous

vertex deliveries and of these one is alive and well. There was one neonatal death and there were 6 stillbirths.

Initially the policy of delivery was one of planned Caesarean Section except for those cases in which there was an intra-uterine death, when labour was induced and a spontaneous delivery followed. The one survivor which was born by vaginal delivery followed spontaneous premature rupture of the membranes. In recent cases, not in this series, a policy of induction with a fixed induction-delivery interval is being followed. This is an attempt to reduce the risks of Respiratory Distress Syndrome to which the premature baby delivered by Caesarean Section is particularly prone. Respiratory Distress Syndrome proved a lethal complication in only one of the present cases but it was a minor complication in 3 other cases.

GENERAL DISCUSSION OF INTRA-UTERINE TRANSFUSION

Where severe Haemolytic Disease of the Newborn is suspected from a previous history and amniocentesis findings, intra-uterine transfusion would seem to have a definite use in enabling the obstetrician to delay delivery until the foetus is of a maturity which has a good chance of survival. Neonatal mortality for premature babies unaffected by Haemolytic Disease of 32 weeks' gestation is of the order of 45% (Crosse, 1957). This must inevitably be increased when Haemolytic Disease is of such severity as to warrant delivery as early as 32 weeks. It is in this early group of affected foetuses that intra-uterine transfusion can play a vital part in the management. Stillbirth or neonatal death occurring at a later maturity - at 35-37 weeks - can be prevented if the warning signs are recognised and induction carried out. With prompt and adequate treatment these infants have a good chance of survival.

It is well to remember before looking too critically at the survival rate of any series of intra-uterine transfusions that the women concerned were subject to all the hazards of pregnancy which can occur without the complication of intra-uterine transfusion. Indeed it is said that accidental haemorrhage and hypertension are common in the sensitized woman (Zilliacus and Eriksson, 1958).

The babies too are liable to the hazards of labour and the neonatal period. Respiratory Distress Syndrome of the newborn is

a not uncommon complication of the apparently healthy premature infant and even exchange transfusion itself carries a mortality of 1% (Phibbs, 1966).

The maternal attitude to intra-uterine transfusion must also be taken into account before embarking on a planned programme of transfusion. Most women, particularly if they have lost one or more babies, are enthusiastic about any procedure which offers a hope of a live child and will willingly agree to undergo intra-uterine transfusion. Occasionally, however, a woman has not the mental attitude to withstand the uncomfortable and perhaps, to the lay mind, alarming operation, and the clinician must think carefully before embarking on a programme which carries a slight but definite maternal risk. Whatever the patient's decision, and it is hers finally, she can only be given the hope and not a promise of a live child.

The decision as to the type of intra-uterine transfusion to be employed in the present time is simple. All types, apart from the closed technique originally described by Liley (1963) and used with slight modifications by others, carry such a high foetal mortality and risk to the mother that they remain largely experimental.

It is in the group of patients with a previous history of stillbirth and neonatal death that successful intra-uterine transfusion carries most satisfaction to mother and clinician but it is the group in which success is hardest to achieve.

When amniocentesis is carried out earlier than the routine 29 and 31 weeks of pregnancy this is usually done at 24-25 weeks and if deemed necessary a programme of planned transfusion begun shortly afterwards. Apart from the history, antibody titre and zygosity of the husband the deciding factor is the optical density deviations of liquor at 450n lying in the upper zone of a Liley type graph (Liley, 1961). Transfusions are begun at 26 weeks or earlier and the difficulty in technique at this stage may be severe. The foetus is extremely mobile and the target area extremely small at this early stage. Three or more transfusions are necessary before delivery to ensure the foetus is delivered in the best possible condition. The survival rate at 26 weeks or under, however, is only 20% (Queenan, 1969).

In the later group of candidates for intra-uterine transfusion, when there is no history of badly affected pregnancies, the decision for active intervention must be taken on routine amniocentesis results beginning at 29 weeks. This means that intra-uterine transfusion begins about 30 weeks and only one or at the most two are needed for the foetus to reach a maturity with reasonable chance of survival.

Technically intra-uterine transfusion at this stage of pregnancy is a much easier procedure and the foetuses are less affected making the success rate for this group greater. With initial transfusion at 28 weeks or later Queenan (1969) reports a salvage rate of 56%.

It is clear, therefore, that the later the intra-uterine transfusion is carried out the better the results. From 25 weeks onwards the results improve and reach a maximum at 30-32 weeks. The survival rate with initial transfusion at 19-24 weeks is so poor that it is questionable whether it should be attempted. Transfusion initiated at 35 weeks produces good results but in view of the risk involved it again is questionable whether it should be done. Close observation of the foetus at this stage and prompt delivery should ensure an equally good survival rate.

In either group it can be difficult to judge the efficacy of intra-uterine transfusion if the foetus at delivery is judged to be less severely affected than predicted. Strict standards of amniotic fluid values is essential in the decision to transfuse or not. A strict standard may occasionally result in an intra-uterine death but this is preferable to performing intra-uterine transfusions on foetuses which would have survived without it and thus incurring the not insignificant risk of the procedure itself.

Liley indicated in his very early reports on intra-uterine transfusion that the presence of foetal ascites or hydrops would prevent the absorption of blood from the peritoneal cavity. This has later been shown not to be so - at least in the presence of ascites alone (Queenan, 1968) so that the presence of ascites alone does not debar a foetus from a successful transfusion. The presence

of hydrops, however, does not produce such a clearcut picture. Cases of gross hydrops as defined by foetal oedema shown by amniogram, ascites and a severe amniotic fluid deviation at 450n show poor results and success or failure depends on the degree of hydrops before treatment (Lucey, 1969).

As a severe degree of hydrops is usually accompanied by a large placenta the dangers of transfusion are increased and the results may not warrant the risks to the mother.

The object of antenatal care and management is to produce a child which is at least responsive to paediatric care and although the operation of intra-uterine transfusion cannot guarantee this, it succeeds in a percentage of cases where, without it, the outcome would be 100% mortality. Although the percentage success may not be as high as one would like intra-uterine transfusion offers hope and some success to the woman whose reproductive capacity is compromised by Rhesus isoimmunization.

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CHAPTER V

PREVENTION OF RHESUS ISOIMMUNISATION

PREVENTION OF RHESUS ISOIMMUNISATION

In the years which followed the discovery of the Rhesus factor and the full realisation of its effects, thoughts of many workers turned to the possibility of prevention. Initially "prevention" was simply an attempt to ameliorate the effect on the foetus.

One of the first substances used to improve the outlook for the foetus was the Rhesus Hapten. This was introduced by Carter in 1947 as an ethereal extract prepared from red cell stroma and in 1949 she first called this material Rhesus Hapten. A Hapten is a substance which, when combined with a suitable carrier such as protein, can stimulate antibody production in a host and can also react specifically with that antibody, but which without a carrier cannot by itself produce antibodies. Carter's rationale in the use of the Rhesus Hapten was to neutralise the existing antibody in a patient without stimulating the production of more antibody. The preparation called Rhesus Hapten was initially crude but Carter, Williamson, Loughrey and Ingram (1956) reported the successful treatment of 135 patients. The Hapten was given by intramuscular injection in large amounts. Later, Carter and Lewis (1958) described the successful treatment of 17 sensitised patients with orally administered Hapten. In 1959 Carter published a review of her work on the Rhesus Hapten and claimed that, if Rhesus Hapten was given to a Rhesus negative woman at any time after discovery of initial sensitisation and if she had no history of erythroblastotic infants, the success rate was 98%.

She further claimed that if a previously sensitized woman with a homozygous Rhesus positive husband and a history of foetal loss from Haemolytic Disease were given the Hapten before a new pregnancy began success would be 100%. Carter claimed that the use of Rhesus Hapten both between and during pregnancies gave the best results.

In view of the availability of the raw material for the production of Rhesus Hapten - time expired Rhesus positive red cells of any ABO group, from which the proteins are precipitated with alcohol and then the liquid Hapten extracted from the precipitate with Dichloromethane or ether - it is surprising that this form of protective treatment did not become widespread. No other workers reported its use and one is forced to conclude that her claims cannot be substantiated.

Another type of protective therapy which gave moderate encouragement for some time was the use of corticosteroids. These were used in the hope that they might work in one of three ways:-

(1) To suppress the production of maternal antibody.

(2) To prevent sensitisation of the foetal cells by maternal antibody.

(3) To lessen the severity and rapidity of destruction of sensitized foetal cells.

Opinion on the value of corticosteroids varied. Hunter (1954)

claimed that corticosteroids had a definite value but De Costa, Gerbie and Potter (1954) disagreed and found little to recommend the administration of cortisone to Rhesus negative sensitized patients. Wiener (1954) also took strong exception to Hunter's views and made the very valid point that the pattern of Haemolytic Disease varies a great deal in sensitized women. It is accepted that even if a woman loses a baby from the effects of Haemolytic Disease she may have a less affected or unaffected baby in the following pregnancy. Thus an unaffected baby may be explained on the zygosity of the husband if he should be heterozygous, but on many other occasions the explanation of a less severely affected baby is unknown.

It is difficult, therefore, to evaluate properly the use of such substances as corticosteroids in Haemolytic Disease when there are so many unmeasurable factors present and when the use of a substance such as corticosteroid is not without danger in itself. Corticosteroids are no longer used in the sensitized patient.

Another attempt at inhibition of Rhesus antibodies to try to protect the foetus was made by Pearse and Hobel (1964). As ribonucleic acid derivatives were thought to be similar to the terminal chemical group of the erythrocyte antigen structures and could inhibit saline anti D but not albumen anti D and as it is known that glucosamine and galactosamine are components of the polysaccharide blood group antigens they decided to try out the effects of glucosamine. They report only one case where a sensitized woman was given 10 percent glucosamine 900ml. at 27 weeks.

The effect on antibody level was minimal and the patient was delivered of a stillborn foetus at 31 weeks.

The use of phenobarbitone to reduce the risk of kernicterus in the neonate was suggested by Maurer, Wolff, Finster, Poppers, Pantuck, Kuntzman, Cunney (1968) and later by Trolle (1968). This is given to the mother in the last few days of her pregnancy and later to the baby. The rationale is that hepatic enzyme activity is enhanced and there is an increase in Glucuronyl transferase which is necessary for the biosynthesis of bilirubin glucuronamide.

One of the most recent attempts to take positive action during pregnancy to minimise the effect of Rhesus antibodies on the foetus of a Rhesus sensitized patient has been by the use of intensive plasmapheresis with a view to reducing the amount of circulating antibody. Plasmapheresis is a process whereby one or two units of blood are removed from a patient by venesection, the plasma separated and the red cells returned to the patient. This can be done frequently and large quantities of plasma removed. It has the added benefit that large quantities of plasma containing high titres of anti Rhesus (D) can be made available for the preparation of anti Rhesus (D) immunoglobulin. This latter aspect of plasmapheresis will be discussed in detail later.

Powell (1968) used plasmapheresis on a series of eight sensitized women with a poor Rhesus obstetric history. He used it on the basis that a woman with a low antibody titre rarely produces a badly affected baby and attempted to lower the antibody

titre in his patients by removing 2,000 ml. of whole blood daily over a period of 7-12 days. He found, however, that the antibody titres dropped to a limited degree but rose again in 2-6 weeks as did the serum proteins. Powell used adjuvant measures of therapy such as intra-uterine transfusion as well in selected cases so that it is difficult to judge the effect of plasmapheresis per se. Perhaps the most valuable information obtained from his series was that intensive plasmapheresis in pregnancy did not harm the patients.

This work was repeated by Clarke, Elson, Bradley, Donohoe, Lehane and Hughes-Jones (1970). They showed that intensive plasmapheresis lowered the concentration of plasma proteins, including total IGg. and individual antibodies. They estimated the fall in antibody concentration by a method using radio-active anti D gamma globulin and not by the conventional agglutination technique. In general they found that the antibody content mirrored the IGg. level during plasmapheresis.

The eight patients in the series were treated by conventional methods including intra-uterine transfusion so once again it is difficult to estimate the effect of plasmapheresis alone.

It must be emphasised again that most of the measures described are primarily attempts to decrease the effect on the foetus and do not attain real prevention of iso-immunization of the mother.

When the circumstances in which isoimmunisation occurs were realised - these being (1) Repeated blood transfusions (2) Therapeutic intramuscular injections of blood (3) Experimental injection of blood into volunteers (4) Foeto-maternal haemorrhage during pregnancy - thoughts turned to the significance of these factors in relation to maternal isoimmunisation. The obvious primary step of matching Rhesus negative blood for Rhesus negative girls and women before the age of the menopause was taken and therapeutic intramuscular injections of blood to women and children were stopped.

In spite of these measures and the additional attempts to ameliorate the effect of Haemolytic Disease the mortality and morbidity kept complacency at bay and reminded workers that there was need for a more fundamental approach to the basic problem - the prevention of isoimmunisation.

Two important observations had been made before 1960, namely:-

(1) TRANSPLACENTAL HAEMORRHAGE

This is the term used to describe the leak of foetal red blood cells from the placental villi into the maternal circulation. The potential leak is thought to occur most often at delivery. Transplacental haemorrhage will be discussed in detail in the following pages.

(2) THE ABO PROTECTIVE FACTOR

This is the term used to describe the prevention of isoimmunisation

by the rapid destruction of foetal cells in the maternal circulation when they are of an incompatible ABO group. This, too, will be discussed in detail later.

The second observation will be discussed first as most of the detailed work on this was done at an earlier stage though it is dependent on the first observation.

THE ABO PROTECTIVE FACTOR

Levine (1943) noted that among families with children affected by Haemolytic Disease of the newborn due to anti Rh. (D) there was a deficiency of matings in which the husband was ABO incompatible with his wife. This observation has been confirmed by other workers - Nevalinna and Vainio (1956); Levine (1958); Murray, Knox and Walker (1965) and supported by experimental work by Stern, Davidson and Masaitis (1956).

Levine, Burham, Katzin and Vogel (1941) and Levine, Katzin and Burham (1941) originally postulated that for the immunisation of the mother there must be an escape of foetal red blood cells into the maternal circulation. With modern methods of detecting these foetal cells in maternal circulation several workers studied the effect of the ABO protective factor. Cohen and Zuelzer (1967) found a lower incidence of foetal cells in the maternal circulation when the foetus was ABO incompatible with the mother. In similar studies, Finn, Clarke, Donohoe, McConnell, Shepperd, Lehane and Kulke (1961) found no foetal cells in ABO incompatible pregnancies but Fraser and Raper (1962) found foetal cells in 16 out of 100 postpartum ABO compatible pregnancies. Various reasons have been

suggested for this phenomenon - rapid removal of the incompatible cells before the Rh. antigen can effect stimulation (Race and Sanger, 1950), competition for the different antigens of the ABO and the Rhesus blood group systems by the same clones of cells (Stern, Goodman and Berger, 1961) or the sequestration of the ABO incompatible cells at a site unfavourable for anti D production (Mollison, 1967a).

ABO protection is not complete. It has been estimated by Nevalinna and Vainio (1956) that in about 20% of such ABO incompatible pregnancies immunization occurs. In more recent studies Murray, Knox and Walker (1965) estimated that maternal anti A gives about 90% protection while maternal anti B gives only about 55% protection. Ascari, Levine and Pollack (1969) suggested that protection from ABO incompatibility appears to be more likely to fail if the mother is of an ABO group other than group O. This may, however, be partly a reflection of ABO group frequencies.

An additional factor may be that women who are group A or B tend to form the saline agglutinating or IgM anti A or anti B while women who are group O form some albumen agglutinating or IgG anti A and anti B fairly frequently (Rawson and Abelson, 1960). The two immunoglobulin types may render foetal erythrocytes immunologically inert at different times.

Group B foetal cells appear to behave in an unusual way. Murray et al (1965) state that the ABO protection factor is reduced when the foetal blood group is B but indicated that babies which

were group B tended to be relatively mildly affected by Haemolytic Disease. Further evidence of unusual behavior of group B was given by Cohen and Zuelzer (1967). They describe two instances in which group B foetal cells persisted in the maternal circulation for several weeks after massive transplacental haemorrhages, although the foetal cells were ABO incompatible with the mothers and were partially coated with maternal anti B.

Once immunization to the Rhesus factor has taken place, the effect of ABO incompatibility is not clear but several observations have been made. Nevalinna and Vainio (1956) state that after immunization has taken place the ABO factor is no longer protective while Murray et al (1965) found that after immunization Rh. positive infants were less severely affected if the foetus was ABO incompatible with the mother. Vos (1966) found that maternal anti-Rh. (D) titres tended to be lower in these cases where the immunizing pregnancy was ABO incompatible.

The other major observation made before 1960 was that of Transplacental Haemorrhage.

TRANSPLACENTAL HAEMORRHAGE

Two studies by Levine, Burham, Katzin and Vogel (1941) and Levine, Katzin and Burham (1941) suggested that foetal red cells could cross the placenta during pregnancy and induce immunization in the mother. A case of severe neonatal anaemia was described by Wiener (1948) who postulated the cause as a massive haemorrhage

from foetus to the mother. Chown (1954) demonstrated the presence of foetal cells in maternal circulation using a differential agglutination technique. This method, however, is not sensitive enough to detect small numbers of foetal cells and it was not until 1957 when Kleihauer, Braun and Betke described the acid elution technique that the study of foetal cells in maternal circulation became detailed. The test is described in English by Zipursky, Hull, White and Israels (1959) and is dependant on the differential elution of adult and foetal haemoglobin by a citric acid/disodium hydrogen sulphate buffer at a pH of 3.3 approximately. While this test is now widely used it is not entirely satisfactory. It is easily upset by poor blood films, minor changes in pH, temperature, and staining techniques. It also requires considerable technical skill for interpretation. In addition it is known that foetal haemoglobin does occur in adults in certain haematological disorders and in such cases foetal type cells can be demonstrated in the circulation by the acid elution technique (Zipursky, Neelands, Pollock, Chown, Israels, 1961). Foetal type cells were also found by Cohen, Zuelzer, Gustafson and Evans (1964) in 5 of 145 non-pregnant blood donors using the acid elution technique.

The other methods of estimating the presence of foetal cells, while perhaps more accurate, are difficult and time-consuming e.g. mixed agglutination technique (Jones and Silver, 1958) and immunofluorescence (Cohen and Zuelzer, 1964).

Many workers confirmed the original theory of Levine of trans-

placental haemorrhage and its influence on immunization (Finn, Clarke, Donohoe, McConnell, Shepperd, Lehane and Kulke, 1961; Taylor and Kullman, 1961; Fraser and Raper, 1962; Zipursky et al, 1963; Finn, Harper, Stallings and Krevans, 1963) and work to investigate the timing of transplacental haemorrhage in relation to immunization plus the effect of obstetric techniques in relation to transplacental haemorrhage now commenced.

SIGNIFICANCE OF FOETAL ERYTHROCYTE IN MATERNAL BLOOD

Although foetal cells were found by many workers (Finn et al, 1961; Taylor and Kullman, 1961; Fraser and Raper, 1962; Zipursky, Neelands, Pollock, Chown and Israels, 1963) a little doubt still remained as to their significance on a test based on elution of adult haemoglobin. It was known that foetal haemoglobin occurred in adults in certain haematological disorders and in such cases foetal type cells could be demonstrated in the circulation by the acid elution technique (Zipursky, Neelands, Pollock, Chown, Israels, 1961). This test is dependent on the alkali resistant nature of foetal haemoglobin (Haemoglobin F).

Rucknagel and Chernoff (1955) measured the level of alkali resistant haemoglobin in pregnant women by immunological methods and found a rise in haemoglobin F was maternal. Bromberg, Salzberger and Abrahamov (1957) found high levels of foetal type haemoglobin in patients with a hydatidiform mole. They too, suggested that the foetal haemoglobin was maternal in origin.

Experimental support to the theory that the foetal type cells in maternal circulation were of foetal origin was given by Zipursky, Neelands, Pollock, Chown, Israels (1962) who showed that foetal cells injected into volunteers appeared identical to those seen in mothers. They found further that these cells, after injection, could be identified in the expected concentration and recognised for as long as 110 days. Similar findings were reported by Finn, Harper, Stallings, Krevans (1963). Borst-Eilers (1960) further reported that the foetal type cells demonstrable by the acid elution technique have the blood group antigens of the foetus and not of the mother.

TIME OF TRANSPLACENTAL HAEMORRHAGE

Everyone who has examined the blood of pregnant women looking for foetal cells has found them to be present in a proportion of the women (Chown, 1968). It has been shown that transplacental haemorrhage may occur during pregnancy and also during delivery (Taylor and Kullman, 1961; Fraser and Raper, 1962; Zipursky et al, 1963; Kreiger, 1966).

It is thought that small transplacental haemorrhages occurring during pregnancy in the unsensitized women may produce a stage of priming or sensibilization, the effect of which, is that if there is a further transplacental haemorrhage in a following pregnancy, the woman will show the full effect of immunisation - that is antibodies will be found in her blood and the baby affected to some degree with Haemolytic Disease. That such a state may occur, with small frequent bleeds producing detectable immunisation and an affected foetus in a first pregnancy has been shown to occur with varying frequency - 2% according to Chown (1968) and 6.5% according to Godel, Buchanan, Jarosch and McHugh (1968).

The most usual method of immunisation is that following a larger transplacental haemorrhage and this is commonest during delivery (Woodrow, Clarke, Donohoe, Finn, McConnell, Sheppard, Lehane, Russell, Kulke, Durkin, 1965 and Woodrow and Finn, 1966).

TRANSPLACENTAL HAEMORRHAGE IN RELATION TO OBSTETRIC MANOEUVRES

Pregnancy and labour are regarded as normal physiological conditions, but certain procedures may be necessary which produce

an element of disruption of foetal vessels leading to an escape of foetal blood into the maternal circulation. Zipursky et al (1963) indicated that Caesarean Section, manual removal of the placenta and amniocentesis lead to an increased risk of foetal blood leak. Taylor and Kullman (1961) showed an increased incidence of transplacental haemorrhage following delivery by forceps and Fraser and Raper (1962) showed an increase in the incidence of foetal cells in the maternal circulation following antepartum haemorrhage.

External cephalic version has also been incriminated in increasing foetal maternal haemorrhage (Vos, 1967 and Pollock, 1968).

To these should be added the risk of transplacental haemorrhage following abortion. Significant amounts of foetal cells have been found in the maternal circulation following therapeutic abortion using various methods of termination (Walsh and Lewis, 1970 and Parmley, Montague and Miller, 1970). The risk is said to be less in threatened or incomplete abortions where there is less trauma (Katz, 1969). The early abortion, before 12-16 weeks, is also said to carry a very small risk (Voigt and Britt, 1969). This will be discussed later in relation to the question of the use of anti Rh. (D) gamma globulin.

The relationship of transplacental haemorrhage to immunisation was shown by Finn, Clarke, Donohoe, McConnell, Sheppard, Lehane, Kulke, (1961) who demonstrated that in women with a "large" transplacental bleed there was a significant association with antibody

formation. They used the Kleihauer technique to assess foetal type cells in maternal circulation. They established the sensitivity of the technique by comparing two blood smears from each of 50 men with an equal number of blood smears from post-partum women. Each smear was scanned twice for three minutes and the number of refractile cells counted. Each individual was given a quantitative figure (foetal score) by taking the average of the number of refractile cells counted in four scores. No male score was found to exceed 0.5. To give a wide margin they accepted a figure of two or more as indicating a definite trans-placental haemorrhage.

In the application of the technique to the series of postpartum specimens in the 1961 series of Finn et al of Rhesus negative women, only one smear was made and the foetal score was the average of two three-minute screens.

In order to establish a correlation between foetal scores and quantity of foetal blood in the maternal circulation mixtures of adult and foetal blood were made at varying dilutions. Thus at 1 in 5,000 the score was 5 (S.D. $\pm$ 1.5). On the assumption of a maternal blood volume of about 5 litres a score of 5 would, therefore, represent about 1 ml. of foetal blood in the maternal circulation.

Clarke (1967) later indicated that the foetal cell score was the number of foetal cells found in 50 low-power fields and roughly assessed that a score of 1-4 implied less than 0.25 ml. of foetal blood in the maternal circulation. He indicated that a score of 5-60 meant approximately 0.25-3 ml. of foetal blood and a score of over 60 meant more than 3 ml. of foetal blood. Clarke indicated

that over 0.25 ml. of foetal blood was significant. The significant score tkaen as 5 or more foetal cells per 50 low-power fields. A small bleed is regarded as 1-4 foetal cells per 50 low-power fields. A more accurate method of scoring is to relate the number of foetal to the number of adult cells and this is the method used in the series presented in the later part of this chapter.

The difficulties of the Kleihauer technique have already been mentioned (Page 141) but in the hands of the Liverpool workers the technique appears to give good results and their use of it in investigating transplacental haemorrhage and its relation to immunization began the modern era of prevention of Rhesus Haemolytic Disease.

EXPERIMENTAL WORK IN PREVENTION OF RH. (D) IMMUNIZATION

Finn et al (1961) injected Cr-51 tagged Rh. positive cells into Rh. negative male volunteers and in half the number of volunteers, followed this with an injection of 10 ml. serum containing anti Rh. (D). They showed that the anti Rh. (D) coated a good proportion of the Rh. positive cells and caused the elimination of at least 50% of the tagged Rh. positive cells in two days.

Clarke, Donohoe, McConnell, Woodrow, Finn, Krevans, Kulke, Lehane and Sheppard (1963) carried out further similar experiments with Rh. negative male volunteers, injecting them with Rh. positive cells and following this with an injection of serum containing anti Rh. (D) antibody. This was a saline agglutinating or complete antibody and they found that, compared with control subjects, antibody formation was enhanced rather than prevented when saline (complete) anti Rh. (D) was

used after the injection of ABO compatible Rh. positive blood into the volunteers. They also noted that in some cases the injected cells survived in the circulation for over a week although, at that time, free saline (complete) anti Rh. (D) could be detected in the recipient's serum.

When Clarke et al (1963) used 35-50 ml. of plasma containing predominantly albumen (incomplete) antibody they found that only 3 out of 21 treated men (14.3%) developed immune antibodies after three to four stimuli compared with 11 out of 21 "control" men (52.4%).

The results of this study suggested that a high percentage of the injected cells had to be cleared from the circulation within 24 hours if immune antibody production was to be prevented. In addition, Clarke et al (1963) postulated that no complete antibody should be present and as high a titre of incomplete anti Rh. (D) as possible should be given and that the antibody should be present in excess.

INTRODUCTION OF ANTI RH. (D) GAMMA GLOBULIN

Throughout all these experimental studies the workers were aware of the danger of the injection of whole blood and plasma - namely that of the transmission of homologous serum hepatitis to the volunteers.

The donor of the blood in all cases was one who had given many times and whose donations had never been implicated in the development of jaundice in the recipients. The same safeguards applied to the plasma containing anti Rh. (D) but the risk remained. Clarke et al (1963) suggested the use of anti Rh. (D) gamma globulin by intramuscular

injection in place of intravenous plasma as formerly. This plasma fraction is free from the risk of transmitting serum hepatitis.

Freda, Gorman and Pollack (1964) successfully used a preparation of anti Rh. (D) gamma globulin to prevent immunization using a series of Rh. negative male volunteers. This was similar to the studies by Clarke et al (1963). Nine Rh. negative male volunteers were challenged monthly for five successive months with 2 ml. of Rh. positive red cells. Four of these volunteers were protected by intramuscular injections of 5 ml. of anti Rh. (D) gamma globulin twenty-four hours before the antigenic challenge. Three months after the last injection the passively acquired Rh. antibodies were no longer demonstrable in any of the four protected volunteers. There was no sign of active antibody production six months after the last injection. Four of the five controls, however were strongly sensitised.

Woodrow, Clarke, Donohoe, Finn, McConnell, Sheppard, Lehane, Russell Kulke, Durkin (1965) carried out a further series of studies and presented evidence to support two important facts:-

(1) That foetal Rh. positive ABO compatible blood could be cleared from the circulation of Rh. negative women volunteers (who were nulliparous and postmenopausal) by the injection of 5 ml. of anti Rh. (D) gamma globulin given intramuscularly.

(2) That transplacental haemorrhage occurred mainly just before or during delivery and that the size of the transplacental haemorrhage was related to the incidence of antibodies detected six months after delivery.

Woodrow and his colleagues now embarked on a clinical trial using

anti Rh. (D) gamma globulin on Rh. negative primigravidae who were at most risk of developing antibodies, that is, women who were delivered of an Rh. positive ABO compatible foetus and who showed evidence of a transplacental haemorrhage using the Kleuhauer method of detecting foetal cells (Prevention of Rh. Haemolytic Disease - a combined study, 1966).

Workers in this combined study examined a series of 156 Rh. negative primigravidae shown to be at risk by virtue of the foetal cells detected in their circulation after the delivery of an Rh. positive ABO compatible baby. Half of these patients were given 5 ml. of anti Rh. (D) gamma globulin not later than 36 hours after delivery, the other half serving as controls. Six months following the injection no immunization had developed in the treated patients but 19 of the 78 controls had become immunized (24.3%). Similar studies have been made by many workers - (Schweider and Preisler, 1966; Robertson and Holmes, 1969; Freda, Gorman, Pollack, Robertson, Jennings and Sullivan, 1967).

Initially 5 ml. of anti Rh. (D) gamma globulin was used but in the combined study (1966) it was suggested that 1 ml. might be enough. Later as Clarke (1967) pointed out per millilitre was a meaningless way to judge the quantity of anti Rh. (D) given. Hughes-Jones (1967) by means of a labelling procedure was able to estimate the quantity of anti Rh. (D) present in gamma globulin in terms of ug. By testing material similar to that used in the Combined Study (1966) Clarke estimated that the amount of anti Rh. (D) used was in the order of 1,000 ug./5ml. and suggested that a lower dose might be as effective. Dudok and De Wit, Borst-Eilers, Weerot and Kloosterman (1968) showed that a dose of 250 ug. of anti Rh. (D) gamma globulin could protect against immunization. At the International Symposium on the Rh. problem in Milan (1969) Finn indicated that a 200 ug. dose was a safe suppressive amount of anti Rh. (D) gamma globulin.

THE SUPPLY OF ANTI RH. (D) GAMMA GLOBULIN

Once it had been shown that isoimmunisation of the Rh. negative pregnant woman could be prevented, the next most important problem in this field was a supply of raw material and hence gamma globulin.

There are several potential sources of anti Rh. (D) gamma globulin:-

(1) From individuals who have been immunised by transfusion.

This is fortunately a very rare occurrence in modern transfusion practice and the amount of raw plasma obtained from such individuals is very small.

(2) From women immunised by pregnancy.

This is the largest source of plasma containing Rh. antibodies. It is, however, one which will become less common as a programme of prevention of immunisation is carried out. Many of these women have become keen blood donors after pregnancy since they have themselves experienced the anxiety and sometimes tragedy of Haemolytic Disease in their children. The high titres which occur during pregnancy, however, are seldom sustained over a long period and in time the blood of such women has little antibody present. Attempts have been made to reduce high levels of antibody by plasmapheresis during pregnancy (Page 135) and this produces a source of high titre plasma. This has not yet become widespread in practice.

While many women immunised by pregnancy give generously of their blood to produce gamma globulin this population is often not the most suitable to use. Apart from the obvious drawback of those who have a history of jaundice many of these mothers tend to have a history of anaemia or to be unavailable for blood donation because of domestic commitments. Many, too, are reluctant to give blood particularly if they have had a difficult confinement. If these women do donate blood it should be taken by plasmapheresis rather than a straight donation as this method yields a larger amount of anti Rh. (D) containing plasma and also avoids depleting the donor of red blood cells.

(3) From postmenopausal or sterilized women

These are women who have usually been sensitized by a pregnancy and whose antibody has been boosted by an injection of Rh. positive red cells. These women are volunteers and to them apply the same ethical and legal problems as apply to Rh. negative male volunteers. The injection of Rh. positive red cells carries a very small but definite risk of homologous serum jaundice. Before this injection can be given these women must be medically examined and the implication of the injection of blood explained to them. Adequate legal arrangements must be made to compensate these volunteers and to cover the Medical Officer who gives the injection, if jaundice should occur. It is also essential to ensure that these women should be given carefully matched blood should they require transfusion in the future. There is liable to be particular difficulty about this if they should move to another country where blood transfusion techniques are not highly developed. A further difficulty

arises in those women who are sterilized and yet of childbearing age. If, by any chance, another pregnancy should occur these women will need to be offered termination of pregnancy.

(4) From Rh. negative male volunteers

These are Rh. negative males who have volunteered to be immunised by the injection of Rh. positive cells in order to stimulate the formation of antibodies. The same legal and ethical problems apply to these volunteers as to the female volunteers mentioned in the previous paragraph.

The risk of transmission of homologous serum jaundice as a result of the injection of red cells can now be reduced for these volunteers and for the women volunteers by testing the blood used for the presence of hepatitis associated antigen.

QUANTITY OF ANTI RH. (D) GAMMA GLOBULIN REQUIRED

Initially plans were made to treat all primigravidae at greatest risk. In Scotland it was calculated from the frequency of the genes concerned that 11,500 mothers per year would be Rh. negative and bear Rh. positive ABO compatible children using the 1964 number of live births. This figure is made up of 2,700 primigravidae and 8,800 multigravidae. These figures are approximations.

If primigravidae alone were treated then on the assumption that 1 ml. of 10g.% immunoglobulin solution would be effective in clearing red cells and provide protection from immunization it was calculated that 85 litres of anti Rh. (D) containing plasma would be necessary and that this could be obtained from 340 donations per year.

If plasmapheresis were used and, assuming that each donor with acceptable antibody titres gave approximately 12 litres of plasma per year, by giving a double donation at two-weekly intervals, it was calculated that 7 such donors would suffice. This would be the minimum number possible and since it would be unlikely that these donors would always be available throughout the year, a more practical number would be 14 donors.

A similar calculation to that above shows that to prevent sensitization of all mothers, regardless of parity, who gave birth to ABO compatible Rh. positive infants in Scotland annually, some 280 litres from 1,120 normal donors would be needed annually. Assuming that this amount of plasma could be obtained by plasmapheresis of suitable donors this could be obtained from a pool of 46 donors. These calculations do not take into account the extension of prevention to abortions and ABO incompatible pregnancies.

Before the introduction of prophylactic anti Rh. (D) gamma globulin, blood from sensitized donors was collected in the normal way from approximately 1,000 donors per year in this region. This was used as anti serum for laboratory purposes only. When the need to increase the yield of antibody containing plasma became evident the collection of such material was tackled more vigorously. Authority to sensitise or boost volunteers was not readily available and the best use had to be made of such sources as were available.

This was tackled initially by approaching women who had been pregnant recently or in the past and who were known personally to the author. With a few exceptions these ladies responded enthusiastically when the situation was explained to them. Where there had been an interval of some time, however, since the previous pregnancy the antibody titres were, in some instances, very low. A further selection of donors was made by going through the old antenatal files of The Regional Transfusion Centre and inviting known sensitized women to donor sessions or offering them "home bleeds" i.e. a Medical Officer going to their homes and carrying out the venesection there. Eventually, when contact was made with many of these women, the lists were computerised and a regular call instituted to sensitised donors in all parts of the Western Region to attend local donor sessions. At this time plasmapheresis was not used. All maternity hospitals in the region were encouraged to notify The Regional Transfusion Centre of their sensitised cases and invited to obtain donations from their patients in the hospital concerned and to encourage them to become blood donors regularly.

In January, 1968 a group of sensitized women known to the author were invited to take part in a programme of plasmapheresis. This group has been gradually expanded and there are now 80 women who give blood regularly by plasmapheresis.

As stated previously these women are a group who do not often have much free time by virtue of their domestic commitments and at present they attend only at two-monthly intervals.

PLASMAPHERESIS

This has been mentioned briefly on Page 13. It consists of withdrawing 500 ml. of blood by venesection into a plastic pack, which has a transfer pack attached, and thereafter keeping the vein open by infusing normal saline. The blood is centrifuged for 15-20 minutes at 1,000 rev./min. in a mistral centrifuge and then the supernatant plasma is separated into the transfer pack. The packed cells are then returned to the donor and the process repeated. It is by far the best way of obtaining a good yield of plasma - about 500-600 ml. from each double plasmapheresis. In addition, because there is little risk to the donor developing anaemia, since the cells are returned to the donor, donations can be given much more frequently than the straight donation of whole blood. Plasmapheresis carries the same risks as a straight donation of blood - infection, air embolus, vaso-vagal attacks, but with a careful technique these can be reduced to a minimal level. Haemoglobin levels are checked at every visit and if the process is carried out intensively, as it can be, plasma proteins should also be carefully observed. The double plasmapheresis takes about one and a quarter hours.

Plasmapheresis is not without problems, other than the obvious medical ones. In this region those donors volunteering for plasmapheresis may need to spend an entire morning or afternoon away from home because of the distance between their homes and The Regional Transfusion Centre. All of these donors are housewives, a few are working as well, and all have young families and domestic ties. In many cases they need to make elaborate arrangements to have children looked after and those who are working need to arrange with their employers to be released from work to come to the Centre. In short, these donors may be put to considerable personal inconvenience in order to give blood.

The idea of monetary payment to volunteer blood donors has never been acceptable in Britain except where there is proven loss of earnings. These special donors are in the same category - that is, they give their blood on a voluntary basis. It is, therefore, of the utmost importance that these women are provided with comfortable transport facilities by The Transfusion Centre and that the plasmapheresis takes place in pleasant non-clinical surroundings. A special room for this purpose has just been provided at The Western Regional Transfusion Centre. In time, it is hoped to provide comparable accommodation and supervision for children so that they may accompany their mothers if necessary.

It is important, too, that a close personal contact is maintained between these donors and the transfusion staff who look after them. To achieve this purpose it is desirable that the same

members of staff should be involved with these donors each time they donate blood. It can now be seen that the results of the use of anti Rh. (D) gamma globulin justifies the slight risk and considerable inconvenience to these donors, but at the same time, great tribute must be paid to these altruistic women who give so generously of their time and their blood to help others.

It is hoped that shortly permission will be given by The Scottish Home and Health Department to boost a selected panel of women, immunised by pregnancy, and who are now either post-menopausal or have been sterilised. These women will be carefully selected, the risks of boosting explained to them, have a full medical check-up and be given an opportunity to discuss the procedure with their families and The Regional Transfusion Staff before the injection is given. These women would then undergo intensive plasmapheresis for some months and then be rested. This would enable the number of plasmapheresis donors to be cut down, although it would be advisable to maintain an adequate number of non-boosted women for plasmapheresis to offset the drop out of any of the boosted donors for one reason or another. In any case, in terms of public relations, having persuaded a woman to take part in an ordinary programme of plasmapheresis it would be inadvisable to discontinue inviting her without a sound reason. It would not be prudent to say that her blood was no longer of value since even low titres of anti Rh. (D) are used to dilute the concentrated preparation. Until the number of sensitized women is drastically reduced by prophylaxis it might, however, be enough to invite women with high titres to give blood 4-6 times after their pregnancy to supplement the pool of plasma from boosted female donors.

As yet there is only one scheme in Scotland whereby Rh. negative male volunteers are immunised. Cook (1969) has successfully immunised 16 Rh. negative male volunteers. Preliminary studies of the yield of raw plasma obtained by plasmapheresis from these volunteers suggest that enough anti Rh. (D) gamma globulin to supply most of Scotland's needs can be produced from this source. On first impression this would seem to remove the need to boost pregnancy-immunised women but it must be remembered that several of these donors may opt out of the scheme for one reason or another and that the availability of a second source of supply is advisable. By making use of all accessible sources and increasing the supply of anti Rh. (D) gamma globulin it is hoped that in time prophylaxis may be available to all Rh. negative women at risk regardless of parity. It is doubtful whether Rhesus Haemolytic Disease can be completely eliminated as will be seen from the results of the use of anti Rh. (D) in the series presented later, but it must eventually come into the category of an uncommon disease.

PRACTICAL TRIAL OF ANTI RH. (D) IMMUNOGLOBULIN

Since anti Rh. (D) immunoglobulin can, at the present time, be prepared only from human plasma containing anti Rh. (D) and its preparation is costly and time consuming, it is essential that its use should be adequately controlled. This means a close liaison between laboratory and clinicians and, while on paper this seems simple, in practice it can be very complicated. This is illustrated by the following description of initial use of anti Rh. (D) gamma globulin in Lanarkshire.

Towards the end of 1967 a small supply of anti Rh. (D) immunoglobulin was made available for use in Lanarkshire. The object of the trial of this material was to try and determine the smallest effective dose of immunoglobulin. The material consisted of 360 vials of 2 ml. of an immunoglobulin solution of varying microgramme content. There was an equal number of vials, each containing, 20, 50, 100 and 200 microgrammes of anti Rh. (D) gamma globulin. The actual dose given was not known at the time of injection.

It was decided to limit the use of this material to the two main maternity hospitals - Bellshill Maternity Hospital and The William Smellie Memorial Hospital. It was further decided that the material would be issued from The Regional Transfusion Centre and that all laboratory tests would be performed there. As each of these hospitals was situated 13 and 8 miles respectively from The Transfusion Centre a good deal of preliminary planning was necessary in order that a workable and efficient trial might be carried out.

Initially, discussions on planning took place between the Senior Obstetricians concerned and The Transfusion Centre ataff and then The Senior Nursing Staff were asked to take part. It was anticipated that the supply of material would last for approximately two years and to avoid the difficulties of changes in Junior Medical Staff during this period it was felt that the permanent Senior Nursing Staff should be invited to co-ordinate the trial within their own hospitals with consultant supervision. This they agreed to do. General Practitioners in the district were informed of the availability of the material within the Specialist Hospitals and invited to send their Rh. negative primigravid patients to hospital for delivery.

It was decided that the material should be given only to patients who fulfilled the following criteria:-

(a) Rhesus negative and primigravid
(b) Delivered of a Rhesus positive ABO compatible foetus
(c) Married
(d) Caucasian

Administrative arrangements within the hospital were made so that a small supply of gamma globulin, issued from The Transfusion Centre, would be kept in the labour-room of each hospital in charge of the labour-room supervisor or her deputy. This material was to be given to a patient on telephoned instructions to each supervisor from The Transfusion Centre following examination of maternal and cord blood. This injection had to be given within 36 hours of delivery.

To allow a proper selection of cases to be made, each Rhesus

negative primigravida who was married and caucasian, had her case sheet starred distinctly on admission to the labour-room and her name entered in a Special Register. Following delivery of the baby, specimens of maternal and cord blood (clotted and unclotted blood from each) were collected by the midwife in charge of the delivery and placed in a special labelled box in the labour-room.

Because of the distances involved between each hospital and The Transfusion Centre, and to avoid delay in examination of the blood specimens, transport from The Transfusion Centre called at each hospital twice daily - at 8.30 a.m. and 3.30 p.m. - to collect the specimens. This enabled all specimens to be examined and results 'phoned well within the time limits and prevented them having to be examined as emergencies.

At weekends one daily collection was made of specimens - once on Saturday and once on Sunday.

A group of senior laboratory technicians took part in the laboratory examinations. Apart from the weekends this was done within normal working hours. The examination of blood carried out included testing for the presence of anti D in the maternal blood, a check on the ABO and Rh. group, and a Kleihauer count. The ABO and Rh. group of the foetal blood were determined and a haemoglobin estimation made on both baby and maternal blood. The technician who performed the blood tests was responsible for telephoning the nominated Senior Sister in each hospital to indicate whether a particular patient should be given the injection or not. The results of all tests, and whether the injection was to be given or not, were recorded on routine rhesus screening forms

and also on a Special Register kept in The Transfusion Centre.

The actual injections were administered by a nominated Senior Sister in each hospital and this entered into the Register of patients entered in the trial and which was kept in each labour-room. The number of each vial and time of injection was also entered in each patient's case sheet.

A separate card, containing - name, address, age, blood group, and later obstetric details of delivery was also placed in each case sheet. To this was attached the number of each vial used. Prior to injection, each patient was given a leaflet explaining the purposes of the injection and explaining the importance of follow-up at six weeks and six months. Only one woman out of a total of 802 potential candidates refused to have the injection. Further specimens of maternal blood were taken on discharge and the patient asked to report back at six weeks and six months after delivery to have blood examined for the presence of antibodies.

The follow-up of patients proved the most difficult part of the trial. Even although the patients were given a date and time to report back to the hospital for follow-up many failed to do so. When a patient failed to come she was sent a letter reminding her about her check-up and offering a home visit if necessary to collect the blood. In many cases these letters were ignored. In defence of the patients one must say that the geographical position of the patient's home in relation to the hospital made travelling difficult and there was the added consideration that most had also the care of a young baby.

Eventually after the last of the material was used and a six

month interval had elapsed all the defaulters were again contacted and a series of home visits arranged. This brought the 77 defaulters down to 7 none of whom could be traced.

The planning and carrying out of this trial required considerable liaison and co-operation between the Transfusion Staff and Medical and Nursing colleagues. To all concerned, as one of the co-ordinators of this trial, I would express my gratitude for their help.

RESULTS

These are detailed in Table 1.

As might be expected the number of significant bleeds detected in the ABO compatible pregnancies with both Rhesus positive and negative babies was greater than in ABO incompatible pregnancies. The numbers involved, however, are very small.

TABLE 1

1.12.67 - 5.1.70

TOTAL INJECTIONS GIVEN - 360

RESULTS OF SCREENING 802 CONSECUTIVE RHESUS NEGATIVE PRIMIPARAE FOR TRANSPLACENTAL HAEMORRHAGE

	SIGNIFICANT BLEEDS Over 5 Foetal Cells per 50 Low Power Fields	SMALL BLEEDS 1 to 4 Foetal Cells per 50 Low Power Fields	NO BLEED	TOTALS
BABY Rh Negative ABO Compatible with mother	51 (6.4%)	33 (4.1%)	188 (23.4%)	272 (33.9%)
BABY Rh Negative ABO Incompatible with mother	4 (0.5%)	1 (0.1%)	60 (7.5%)	65 (8.1%)
BABY Rh Positive ABO Compatible with mother	70 (8.7%)	60 (7.5%)	236 (29.4%)	366 (45.6%)
BABY Rh Positive ABO Incompatible with mother	3 (0.4%)	6 (0.7%)	78 (9.7%)	87 (10.8%)
BABY Du ABO Compatible with mother	2 (0.2%)	- -	7 (0.9%)	9 (1.1%)
BABY Du ABO Incompatible with mother	- -	- -	3 (0.4%)	3 (0.4%)
TOTALS	130 (16.2%)	100 (12.4%)	572 (71.3%)	802 (100%)

Three hundred and sixty Rhesus negative primigravid women received an injection of anti Rh. (D) gamma globulin out of a total of 802 considered. Three women have become sensitized and now show the presence of antibodies - 0.83%. This illustrates that anti Rh. (D) gamma globulin does not provide absolute protection in the doses used but does reduce the incidence of sensitization to a very marked degree.

DETAILS OF FAILURES

TABLE 11

Case	Dose of anti Rh. (D) immunoglobulin	Foetal cell count	Time anti-bodies developed	Type and titre of antibody
1	100 ug.	2 cells/ 50 L.P. fields. Not significant	At delivery of second pregnancy	1/2 anti D by enzyme technique
2	200 ug.	502 cells/ 50 L.P. fields. Large bleed	At delivery of second pregnancy	1/16 anti D by I.A.G.T.
3	200 ug.	No foetal cells	At 6th month - second pregnancy	1/8 anti D by I.A.G.T.

The present routine dose of 200 ug. would probably have prevented immunization in Case 1 and a larger dose, taking cognisance of the unusually high foeto-maternal haemorrhage, would probably have prevented immunization in Case 2. There is no apparent explanation for Case 3 but it is possible that the patient may have had undetected foeto-maternal haemorrhages in the middle trimester of her second pregnancy and thus became sensitized.

Twenty of the total of 360 patients who received injections of this anti Rh. (D) gamma globulin have completed a successful second pregnancy by the end of 1969 and have not shown the presence of antibodies. Nine of these twenty cases were again eligible for administration of anti Rh. (D) gamma globulin and were given injections of the standard preparation now in use. None of these patients showed immunization six months after delivery.

Further success in prevention of Rhesus sensitization can be seen in the general use of anti Rh. (D) gamma globulin in The Western Region of Scotland. The use of this, in 200 ug. doses, has not been quite so rigid as in the initial Lanarkshire series. While the use was confined to Rhesus negative women who delivered ABO compatible Rhesus positive babies, race and marital status were not defined. Many laboratories were involved in the blood examinations, each with its own standard, and there was no central control.

The results of the use of anti Rh. (D) gamma globulin in the Western Region for the period 1st March, 1968 - 31st October, 1970 are shown overleaf.

RESULTS OF GENERAL USE OF ANTI RH. (D) GAMMA GLOBULIN IN WEST OF SCOTLAND REGION

(Survey from 1st March, 1968 - 31st October, 1970)

Total No. of injections	-	1,671	
No. without detectable antibody 6 months after delivery	-	1,643	(98.33%)
No. with detectable antibody at 6 months after delivery	-	22	(1.31%)
No. with possible antibody present 6 months after delivery	-	6	(0.36%)

OBSERVATIONS ON 22 FAILURES

A foetal cell count was performed on only 4 of the failures. No foetal cells were found in these 4 examinations. Five women, on close questioning after the appearance of anti Rh. (D) antibodies in their serum, admitted to a previous pregnancy after which no anti Rh. (D) gamma globulin had been administered. Anti Rh. (D) gamma globulin does not, of course, have any protective action once a patient is sensitized. A further 5 women were unmarried.

CAUSES OF FAILURE OF PROTECTION

Protection against Rhesus sensitization by the use of Anti Rh. (D) immunoglobulin is not absolute. There are several possible reasons for failure. Sensitization is most common at delivery but occasionally a woman is sensitized by transplacental

haemorrhage during pregnancy. In these cases the antibody titre may be too low to be detected and the woman appears to be unsensitized when the anti Rh. (D) immunoglobulin is given. She is, in fact, sensitized and the anti Rh. (D) immunoglobulin cannot reverse the process.

Another reason for failure is that the woman may have a very large foeto-maternal haemorrhage which is not neutralised by the normal dose of anti Rh. (D) immunoglobulin. This is illustrated by Case 2 on Table 11.

A further reason for failure is poor laboratory control and, therefore, the lack of detection of antibody which is present. This is one reason why the use of anti Rh. (D) immunoglobulin must be controlled by a nominated laboratory with high standards. This is particularly true when the material is used in maternity units outwith the specialist maternity hospitals as these non-specialist hospitals often have no laboratory facilities within the particular maternity units. This is illustrated by the results in the use of National Health Service in The Western Region of Scotland when not all pre-injection laboratory procedures were carried out e.g. foetal cell counts. In widespread use, too, very rigid selection of patients may not be possible and apparently potential candidates may not admit to previous pregnancies. This, too, is shown in the results of the use of National Health anti Rh. (D) gamma globulin.

MATERNAL RISKS OF ANTI RH. (D) IMMUNOGLOBULIN

The risk of transmission of serum hepatitis was eliminated

with the preparation of the immunoglobulin form of anti Rh. (D) by fractionation procedures (Mollison, 1967b). Local discomfort, sometimes associated with fever of no more than 24 hours duration, has occasionally been reported (Clarke, 1968).

Although systemic reactions are rare, sensitization to gamma globulin has been reported (Clarke, 1968) and this possibility should be kept in mind. If antibodies to gamma globulin are found, anaphylactic phenomenon may occur at some future time when gamma globulin is again given. This is obviously a rare possibility in view of the vast amount of gamma globulin which has been given and the rarity of reports of sensitization. For practical purposes this probably need only concern patients with hypogammaglobulinaemia (Henney and Ellis, 1968). If this problem proves to be significant it may be solved by the use of plasmin treated immune serum globulin (Scouris, 1967).

It has long been recognised that intravenous administration of gamma globulin is dangerous (Barundun, Kistler, Jeunet and Isliker, 1962) therefore the immunoglobulin must be given intramuscularly. It is not advisable to give anti Rh. (D) immunoglobulin to Rhesus positive women although Clarke (1968) reported two cases where the injection was given to two patients who were later found to be Rhesus positive and no reaction occurred. In discussion at the International Symposium in the management of the Rhesus problem in Milan, 1969 Chown reported that two Rhesus positive babies had been given the injection of immunoglobulin intended for their mothers. Apart from developing a weakly positive Coomb's test these infants showed no reaction.

EXTENSION OF THE USE OF ANTI RH. (D) GAMMA GLOBULIN

The trial of the initial material made available for use in the Lanarkshire Specialist Maternity Hospitals ended on 5th January, 1970.

From 1st March, 1968 anti Rh. (D) gamma globulin was made available for general use in Specialist Maternity Hospitals to Rhesus negative unsensitized patients who had no living children and who were delivered of a Rhesus positive ABO compatible child. No differentiation was made on the marital status or race of the patients.

On 15th December, 1969 the use of anti Rh. (D) gamma globulin was extended to include Rhesus negative women without antibodies delivering a second child who was Rhesus positive and ABO compatible. At the same time the material was made available for use in the General Practitioner Maternity Units, provided the necessary maternal and cord blood specimens were examined in a designated laboratory. The effect of this latter extension was to relieve the pressure on Specialist Maternity Hospital beds. Hitherto, all appropriate Rhesus negative patients had to be confined in Specialist Maternity Hospitals and the great majority had normal pregnancies and deliveries which could have taken place satisfactorily outwith these hospitals.

From 1st October, 1970 the use of anti Rh. (D) immunoglobulin has been extended to cover all non-immunised women in first or second pregnancies delivering a Rhesus positive baby regardless of the ABO group. In addition the material is now available for Rhesus negative women without a live child who have an abortion.

In all of these cases the same rigid laboratory control has been carried out with the exception that the Rhesus group of the foetus is not known in early abortions. The Regional Transfusion Centre has been nominated as the laboratory in Lanarkshire where all the specimens of blood are examined. This has meant an extension of the transport arrangements as for the initial use of anti Rh. (D) but as the time limit for injection has been extended to 60 hours in place of the original 36 hours the timing of transport is not quite so critical.

FUTURE USE OF ANTI RH. (D) IMMUNOGLOBULIN

The introduction of anti Rh. (D) is one of the major steps in prophylactic medicine. Although Haemolytic Disease is not a major disease in terms of numbers, in terms of human involvement it is important.

The use of this material is being extended as the raw material becomes available and it seems only logical that it should be extended to cover ALL women at risk regardless of parity. The initial use, restricted to Rhesus negative primigravidae at special risk, ensured her of at least two children unaffected by Haemolytic Disease. When the use was extended to second pregnancies such a patient was ensured of three children unaffected by Haemolytic Disease. Now the use is extended to Rhesus negative primigravidae and secundigravidae who are at less risk by virtue of delivering an ABO incompatible child. In addition patients without a live child and having an abortion are now eligible to be treated with anti Rh. (D) immunoglobulin.

There could be no argument against the use of anti Rh. (D) in the first two groups of patients - the Rhesus negative primigravidae and secundigravidae delivering a Rhesus positive baby. The question of abortion, however, raised problems. The patient having a spontaneous abortion and having no living children should again be treated without argument.

The introduction of the Abortion Act in 1967, however, has caused an increase in the number of abortions per year in all groups of patients and dissention has arisen over the use of anti Rh. (D) gamma globulin in these cases. It is generally agreed that the later the abortion the more risk there is of foeto-maternal bleeding and hence risk of sensitization but many therapeutic abortions are carried out before 16 weeks. Opinion is divided as to how important these early abortions are in production of Rhesus isoimmunization. Murray, Barron and McNay (1970) suggest that there is insufficient evidence to justify blanket administration of anti Rh. (D) immunoglobulin while Freda, Gorman, Galen and Tracy (1970); Edwards (1970); Goldman and Eckerling (1970); Walsh and Lewis (1970) indicate that anti Rh. (D) immunoglobulin should be given to all patients undergoing termination of pregnancy at any stage and by whatever method.

In a series of 917 sensitized cases there were 4 women who became sensitized following an abortion. The time of the abortions varied from 10-26 weeks but it is interesting to note that all 4 have had a history of severe Haemolytic Disease. Two of them had intra-uterine transfusions to procure a live child and indeed one patient has only 2 live children out of 5 pregnancies.

Although the number of patients sensitized by abortion in this series is very small the effect has been so devastating in these cases that it warrants a "prevention if at all possible" approach to the problem. The incidence of abortion will increase as a result of the Abortion Act of 1967 and it is often not easy to obtain a follow-up of these patients but it would seem that they should be given the benefit of anti Rh. (D) immunoglobulin. This is particularly true of the young unmarried girl undergoing termination. If sources of anti Rh. (D) immunoglobulin are properly mobilised the absorption of all women at risk because of an abortion into the recognised categories of patients to be treated is surely justified.

The problem of Rhesus Haemolytic Disease of the Newborn has existed for many years but it seems that now the problem will be on a minimal scale. Much work by many people has gone into the investigation, management and now prevention of this disease. It is perhaps a classic example of the interdependence of clinicians and laboratory worker in finding a solution to one of the mysteries of medicine. The result has certainly been successful in removing the fear of the effects of Haemolytic Disease on the children of many thousands of women.

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TABLE 11

Case No.	1st I.U.T. weeks	No. of I.U.T's	Delivery weeks	Adult Cells %	I.A.G.T.	Cord Blood Hb. gm.%	Bil. mgm.%	Outcome	Treat
1	28½	2	33	N.D.		Haemolysed		S.B.	-
2	32	2	34	N.D.		Haemolysed		S.B.	-
3	28	2	31	80%	+	4.9	4.4	N.N.D.	2 exchanges
4	31½	1	33	N.D.		Haemolysed		S.B.	-
5	32	1	35	20%	+	10.2	6.2	A & W	3 exchanges
6	31	2	34½	50%	+	12.4	7.1	A & W	2 exchanges
7	28	3	37	60%	+	7	6.4	A & W	1 exchange
8	32	1	37	50%	+	7	6.4	A & W	1 exchange
9	29	3	37	70%	+	10.4	4.8	A & W	2 exchanges
10	32	2	34	N.D.		Haemolysed		S.B.	-
11	30	2	35	60%	+	10.6	7.3	N.N.D.	2 exchanges
12	30	1	34	50%	+	9.6	6.6	A & W	3 exchanges
13	31	1	36	70%	+	8.4	8.1	A & W	2 exchanges
14	26	3	35	100%	-	12.4	3.1	A & W	3 top-ups
15	27	2	31	N.D.		Haemolysed		S.B.	-
16	26	3	33	100%	-	13.6	3.4	A & W	2 top-ups
17	30	2	35½	60%	+	10.8	6.4	A & W	3 exchanges
18	30	2	35	80%	+	5.0	4.9	N.N.D.	1 exchange
19	29	2	33	N.D.		Haemolysed		S.B.	-
20	26	1	30	N.D.		Haemolysed		S.B.	-
21	28	1	33½	20%	+	3.2	5.2	N.N.D.	-
22	28	1	29	50%	+	1.8	-	N.N.D.	-
23	28½	3	36½	N.D.		Haemolysed		N.N.D.	-
24	31	3	35	N.D.		Haemolysed		S.B.	-
25	32½	1	35	N.D.		Haemolysed		S.B.	-
26	29	1	31	20%	+	3	4.6	N.N.D.	Partial exchange

KEY
I.U.T. - Intra-uterine Transfusion
S.B. - Stillbirth
N.N.D. - Neo-natal Death
A & W - Alive and well
N.D. - Not done

S U M M A R Y

The object of this Thesis is to present and discuss various aspects of Management and Prevention of Rhesus isoimmunization within a County Obstetric Service which caters for a population of 600,000. The work presented was carried out over the years 1958 - 1968, partly within the County Obstetric Service and partly with the Regional Transfusion Centre.

The first chapter traces the history of Rhesus isoimmunization starting in 1939 with the beginning of the elucidation of the Rhesus factor and its connection with the syndrome of erythroblastosis foetalis. The advances in the knowledge of this factor and its effect are followed throughout the subsequent years. The management of pregnancy and treatment of the newborn are also followed from earl induction of labour based on antibody titre and original exchange transfusion of the baby to the present day assessment by liquor amnii bilirubin content and treatment of the severely affected baby by intra-uterine transfusion. The early attempts at amelioration of the effects of isoimmunization during pregnancy are described and followe to the present use of anti Rh. (D) gamma globulin to prevent the sensitization of the Rhesus negative mother to the Rhesus factor.

The second chapter describes how the pattern of management has evolved over the past eleven years. First came the establishment of special clinics for sensitized patients, followed by the introduction of the diagnostic aid of amniocentesis. This chapter includes a broad picture of the material studied, including tables detailing typ of antibody found, frequency of antibody, ABO relationship of parents and baby and parity. The overall results are presented as represent

the three periods:- (1) pre Rhesus clinic (2) Rhesus clinic and (3) Rhesus clinic plus amniocentesis and show how the perinatal mortality has improved, reflecting the improvement in antenatal care.

The third chapter contains a detailed review of the use of liquor amnii bilirubin content in the prediction of the severity of erythroblastosis of the foetus. The technique and risks of amniocentesis are fully described. One hundred and seventy three cases are presented in detail and correlation made between the liquor amnii prediction and the condition of the baby at birth as judged by the cord haemoglobin and bilirubin. These correlations were not always found to be satisfactory and a better correlation was found between liquor amnii prediction and a suggested Cord Blood Factor obtained from the ratio cord haemoglobin in gms.% divided by the cord bilirubin in mgm.%. (These results are presented in tabular and diagrammatic form).

The fourth chapter gives a detailed review of the use of intra-uterine transfusion of the foetus from the original paper describing this procedure to the present time. The selection of cases, risks to mother and foetus and the present status of intra-uterine transfusion are considered.

Twenty-six pregnancies so treated are fully discussed in detail and the survival rate is shown to be similar to the various published series. The various operative techniques are fully discussed and a short joint publication on technique is included. The cause of perinatal mortality is given when possible for the babies which did not survive.

The final chapter deals with the prevention of Rhesus isoimmunization. It describes the early attempts to ameliorate the effects of immunization on the foetus and later the recent work on prevention of isoimmunization by the use of anti Rh. (D) gamma globulin. The background and experimental work in this field is described. The sources of raw material necessary to prepare anti Rh. (D) gamma globulin are considered and experience in the use of plasmapheresis to obtain this material is described.

A series of cases treated with anti Rh. (D) gamma globulin before the general introduction of anti Rh. (D) gamma globulin is presented and the practical difficulties of administration discussed. The results of its use in a wider series is also presented.

The relevant literature has been reviewed in all chapters and references are shown at the end of each chapter.